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Excision Repair Cross-Complementation Group 6 Gene Polymorphism is Associated with the Response to FOLFIRINOX Chemotherapy in Asian Patients with Pancreatic Cancer

아시아계 췌장암 환자에서 **Excision Repair Cross-Complementation Group 6** 유전자 다형성과 **FOLFIRINOX** 항암화학요법에 대한 반응성에 관한 연구

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Abstract

FOLFIRINOX is currently one of the standard chemotherapy regimens for pancreatic cancer patients, and is known to be more effective in the presence of the BRCA mutation, one of the DNA damage repair (DDR) gene mutations. However, BRCA mutations are less common in pancreatic cancer patients, especially in Asians. We performed a study to discover novel DNA damage repair (DDR) gene variants associated with the response to FOLFIRINOX chemotherapy in patients with pancreatic cancer. We queried a cohort of pancreatic cancer patients who received FOLFIRINOX chemotherapy as the first treatment and who had tissue obtained through an endoscopic ultrasound-guided biopsy that was suitable for DNA sequencing. We explored variants of 148 DDR genes based on whole exome sequencing and performed multivariate Cox regression to find genetic variants associated with progression-free survival (PFS). Overall, 103 patients were included. Among 2384 variants of 141 DDR genes, 612 non-synonymous variants of 123 genes were selected for Cox regression analysis. The multivariate Cox model showed that rs2228528 in *ERCC6* was significantly associated with improved PFS (hazard ratio 0.54, $p = 0.001$). The median PFS was significantly longer in patients with rs2228528 genotype AA vs. genotype GA and GG (23.5 vs. 16.2 and 8.6 months; log-rank $p < 0.001$). This study suggests that rs2228528 in *ERCC6* could be a potential predictor of response to FOLFIRINOX chemotherapy in patients with pancreatic cancer.

Keywords : pancreatic cancer; FOLFIRINOX; DNA repair; *ERCC6*; progression-free survival

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LIST OF ABBREVIATIONS

DNA: deoxyribonucleic acid

DDR: DNA damage repair

PFS: progression-free survival

EUS: endoscopic ultrasound

EUS-FNB: EUS-guided fine needle biopsy

CT: computed tomography

BMI: body mass index

ECOG: Eastern Cooperative Oncology Group

AJCC: American Joint Committee on Cancer

CA 19-9: carbohydrate antigen 19-9

SNP: single-nucleotide polymorphism

INDELs: insertions/deletions

RNA: ribonucleic acid

GVB: gene-wise variant burden

FDR: false discovery rate

HR: hazard ratio

CI: confidence interval

NER: nucleotide excision repair

GGR: global genome repair

TCR: transcription-coupled repair

VAF: variant allele frequency

PARP: poly(adenosine diphosphate-ribose) polymerase

IQR: interquartile range

Chapter 1. Introduction

Pancreatic cancer is a lethal disease with a 5-year survival rate of 9% [1]. One of the reasons for this poor prognosis is that 85–90% of patients are diagnosed at an advanced stage where surgical resection is not possible [1,2]. Therefore, a large number of pancreatic cancer patients receive chemotherapy as an initial treatment, and the two preferred chemotherapy regimens today are FOLFIRINOX and Gemcitabine plus albumin-bound paclitaxel [3–5]. Predicting which of these two chemotherapy regimens will be more effective for each patient helps determine the best first-line chemotherapy. One known factor in this regard is that patients with mutations in DNA damage repair (DDR) genes respond well to FOLFIRINOX. The FOLFIRINOX regimen consists of four drugs: Oxaliplatin, leucovorin, irinotecan, and 5-fluorouracil [4]. Of these, oxaliplatin is a platinum-based anticancer drug that uses gene disruption as a mechanism [6]. In patients with mutations in the DDR gene, the addition of gene disruption by platinum-based chemotherapy leads to cancer cell death by synthetic lethality, resulting in a better response to platinum-based chemotherapy [7,8]. In particular, the BRCA mutation is the best-known DDR gene mutation, and the National Comprehensive Cancer Network guideline recommends the use of FOLFIRINOX in the presence of the BRCA mutation [3]. However, BRCA mutations are not very common in pancreatic cancer patients, occurring at a frequency of about 5% of cases worldwide, and there are reports that the frequency of BRCA mutations is even lower in Asians, at a frequency of less than 1% [9,10]. Therefore, further research is needed regarding whether other variants of DDR genes are associated with a good response to FOLFIRINOX,

especially in Asians. Although a few studies have reported genes associated with FOLFIRINOX chemotherapy responses, those studies were based on targeted gene sequencing and were there-fore limited to the analysis of only a few DDR genes [11,12]. Thus, in this study, we aimed to find novel variants of DDR genes that could predict the response to FOLFIRINOX chemotherapy based on whole exome sequencing analysis.

Chapter 2. Methods

2.1. Patients and Data Collection

Patients were selected from the prospectively collected pancreatic cancer database of the Seoul National University Hospital between May 2017 and May 2019. Patients with the following criteria were included: (i) Histologic diagnosis of pancreatic ductal adenocarcinoma from tissue obtained through endoscopic ultrasound (EUS)-guided fine needle biopsy (EUS-FNB); (ii) locally advanced or metastatic pancreatic cancer; (iii) chemotherapy with FOLFIRINOX regimen as the first line; (iv) available tissue obtained through EUS-FNB for DNA sequencing. Patients who discontinued FOLFIRINOX chemotherapy despite no disease progression in computed tomography (CT) scan (due to patient's refusal, poor performance status, or adverse effects of the FOLFIRINOX regimen) or who were lost to follow-up or transferred out were excluded.

Baseline demographic and clinical data, including age, sex, body mass index (BMI), Eastern Cooperative Oncology Group (ECOG) performance status, primary tumor location, primary tumor size, node involvement, distant metastasis, tumor-node-metastasis stage by the 8th American Joint Committee on Cancer (AJCC) classification [13], baseline serum carbohydrate antigen 19-9 (CA 19-9) level, best response to FOLFIRINOX chemotherapy, surgical resection after FOLFIRINOX chemotherapy, and survival were obtained from medical records. Assessment of chemotherapy response was performed by CT scan every three or four cycles of

chemotherapy according to the Response Evaluation Criteria in Solid Tumor version 1.1 [14]. Written informed consent was obtained from all patients. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University Hospital.

2.2. Sample Acquisition through EUS-FNB and DNA Extraction

EUS-FNB was performed by one of four expert endoscopists with experience of > 1000 cases for pancreatic diseases. Patients underwent conventional EUS-FNB using a linear-array echoendoscope (GF-UCT 240, GF-UCT 260; Olympus Optical Co., Tokyo, Japan) and a 19- or 22-gauge needle (EZ Shot 3; Olympus Medical, Tokyo, Japan, Acquire; Boston Scientific, MA, USA). For histological diagnosis, tissue was obtained through at least two needle passes, and if there was no visible core tissue, up to two more passes were performed. Afterwards, samples for biobanking were obtained through 1–2 needle passes to obtain visible core tissue. These specimens for biobanking were placed in a cryotube and then immediately frozen in liquid nitrogen and stored in a deep freezer at −80 °C until DNA extraction. The HiGene Genomic DNA Prep Kit (GD141-100, BIOFACT, Daejeon, Korea) was used for DNA extraction.

2.3. Whole Exome Sequencing and Variant Calling

Exome sequencing was conducted using the Ion AmpliSeq Exome panel (A29854, Thermo Scientific, MA, USA) to screen the entire genome's coding sequence regions. Sequencing libraries were prepared using the Ion Ampliseq Library Kit Plus (A29854, Thermo Scientific, MA, USA). Libraries were quantified using the Agilent 2100 Bioanalyzer (Agilent, CA, USA) and then diluted to about 100 pM. Subsequently, 50.0 μL of the barcoded libraries were combined into sets of two barcodes. The combined libraries were sequenced using the Ion S5XL platform with 540 Chip (A27766, Thermo Scientific). Torrent Suite Software v5.0.2 was employed for generating mapped reads to the human reference genome build (hg19) with germ-line and low-stringency settings. Single-nucleotide polymorphism (SNP) variants and short insertions/deletions (INDELs) were identified using the Genome Analysis Toolkit 2.8-1 UnifiedGenotyper [15] and Torrent Variant Caller plugin v1.0.0. Raw reads were aligned to the reference human genome (hg19/GRCh37). We manually reviewed the sequence alignment of whole variants by IGV 2.8.9 [16] to exclude false-positive variant calls. All called variants were annotated using ANNOVAR [17]. We set up the DDR genes to be analyzed with reference to the previous study [18] (Table 1), and only non-synonymous variants of these DDR genes that have the potential to affect gene function were included in the further analysis.

2.4. Statistical Analysis

Continuous data are shown as median and interquartile ranges, whereas categorical data are shown as number and percent. Progression-free survival (PFS) was defined as the interval between the start date of FOLFIRINOX and the date of disease progression or death. PFS was assessed using the Kaplan–Meier method and the log-rank test. A p-value < 0.05 was considered significant. Multivariate Cox proportional hazard regressions adjusted for clinical variables were performed to evaluate the association between PFS and each of the genetic variants of DDR genes. The adjusted clinical variables were age, sex, tumor–node–metastasis stage, tumor location, body mass index, serum CA 19-9, and surgical resection after chemotherapy. For genetic variants showing a significant association with PFS, RNA expression analysis was performed using data from the 1000 Genomes Project [19], and Student's t-test was used for this analysis. In addition, synthetic association analysis was performed on genes adjacent to genes showing a significant association with PFS, and gene-wise variant burden (GVB) calculation [20], linear regression with permutation, and Fisher's exact test were appropriately used for this analysis. Benjamini–Hochberg multiple testing correction was applied to estimate the false discovery rate (FDR). An FDR-adjusted p-value < 0.1 was considered statistically significant. All statistical analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA), MedCalc Statistical Software version 19.6.1 (MedCalc Software Ltd., Ostend, Belgium), and R 3.6.3 (the R Foundation for Statistical Computing, Vienna, Austria).

Chapter 3. Results

3.1. Patient Characteristics

During the study period, 304 patients were diagnosed with pancreatic cancer through endoscopic ultrasound EUS-FNB. Of these patients, we excluded patients who did not meet the inclusion criteria (n = 99), discontinued FOLFIRINOX treatment without disease progression (n = 30), or were lost to follow-up or transferred out (n = 72). A total of 103 patients were included in this study (Figure 1).

Baseline characteristics of patients are shown in Table 2. The median age of the study patients was 64 years. All patients had an ECOG performance status of 0 or 1. Metastatic pancreatic cancer was 35.9%. In response to FOLFIRINOX treatment, 32% of patients achieved partial response, 55.3% of patients achieved stable disease, and 12.6% of patients had progressive disease. Surgical resection after FOLFIRINOX treatment was performed in 39.8% of patients.

3.2. Genetic Variants of DDR Genes Predicting PFS

Of the 148 DDR genes we analyzed, a total of 2384 variants were found in 141 genes. Among these variants, there were 612 non-synonymous variants in 123 genes. Multivariate Cox regression adjusted for clinical factors showed that out of

612 non-synonymous variants, only rs2228528 in the gene *ERCC6* was a genetic variant significantly associated with PFS (hazard ratio (HR) 0.54, $p = 0.001$, FDR adjusted $p = 0.08$) (Figure 2).

3.3. Response to FOLFIRINOX According to rs2228528 in *ERCC6*

There are three rs2228528 genotypes in ERCC6: GG, GA, and AA. The number of patients for each genotype was GG (n = 39), GA (n = 41), and AA (n = 23). The response to FOLFIRINOX chemotherapy and the rate of curative surgery after FOLFIRINOX treatment is shown in Table 3. Response rates were not significantly different between rs2228528 genotypes in *ERCC6*. However, the disease control rate was significantly higher in rs2228528 genotype with the A allele in *ERCC6* (76.9% for the GG genotype, 90.2% for the GA genotype, 100.0% for the AA genotype, $p = 0.024$). Surgical resection rates after FOLFIRINOX chemotherapy tended to be higher in the AA or GA genotype than in the GG genotype of rs2228528 in *ERCC6*.

3.4. PFS Analysis According to rs2228528 in *ERCC6* and Factors predicting PFS

The median PFS was 8.6 months (95% confidence interval (CI), 5.8–10.6) for the GG genotype carriers, 16.2 months (95% CI, 10.8–22.4) for the GA genotype

carriers, and 23.5 months (95% CI, 12.0–23.5) for the AA genotype carriers (Figure 3). Multivariate Cox model for PFS showed that rs2228528 genotype with the A allele in *ERCC6* (HR 0.54; 95% CI, 0.37–0.78, $p = 0.001$) and surgical resection after FOLFIRINOX chemotherapy (HR 0.27; 95% CI, 0.14–0.52, $p < 0.001$) were independent predictors for better PFS, whereas metastasis stage M1 (HR 2.21; 95% CI, 1.16–4.21, $p = 0.016$) was an independent predictor for poor PFS (Table 4).

3.5. RNA Expression Analysis and Synthetic Association Analysis According to rs2228528 in *ERCC6*

There was no significant difference in RNA expression according to the genotype of rs2228528 in ERCC6 (Figure 4). There were 12 genes in our data and 192 genes in the 1000 genomes project data that showed significant GVB differences according to the rs2228528 genotype. Among these genes, there were 3 variants in 3 genes in our data, which showed differences depending on the rs2228528 genotype, and one (rs10999147 in *AIFM2*) of those three variants was also found in the analysis of 1000 genomes project data. However, a total of three variants including rs10999147 identified in our data did not show a significant association with PFS (Figure 5) (Table 5).

Chapter 4. Discussion

In this study, we demonstrated that the A alleles of rs2228528 in *ERCC6* were significantly associated with longer PFS in pancreatic cancer patients who received FOLFIRINOX chemotherapy. This suggests that the allele A carriers of rs2228528 in *ERCC6* had a better response to FOLFIRINOX than the allele G carriers. To the best of our knowledge, this is the first study to report that an SNP of *ERCC6* is related to the response to FOLFIRINOX chemotherapy. *ERCC6* plays important roles in the nucleotide excision repair (NER) pathway, one of the pathways in the DDR response [21,22]. The NER pathway repairs DNA damage through steps involving recognition of a DNA damage lesion, unwinding DNA, making incisions around the DNA lesion, and resynthesis and ligation of DNA [23]. DNA damage recognition by the NER pathway consists of two arms: The global genome repair (GGR) branch that recognizes non-transcribed lesions and the transcription-coupled repair (TCR) branch that recognizes transcribed lesions [24]. *ERCC6* is an important component of TCR. If DNA damage in a transcribed gene cannot be repaired due to TCR defects, RNA polymerase II is stalled, which triggers apoptosis [25]. The mechanism of platinum-based chemotherapy is the formation of platinum-DNA adducts followed by intra- and inter-strand crosslinks, which inhibit DNA replication and lead to apoptosis [26]. Since TCR deficiency and platinum-based chemotherapy both involve gene disruption, synthetic lethality can occur if both are present at the same time [7]. Therefore, TCR deficiencies will enhance the response to platinum chemotherapy as demonstrated in an

experimental study using human cells [27]. In addition, as GGR deficiency in the NER pathway did not correlate with the responsiveness of platinum chemotherapy in that study, it appears that TCR deficiency in the NER pathway is more associated with responsiveness to platinum chemotherapy [27]. Consistent with this, our study revealed that patients had a better response to the FOLFIRINOX regimen, which contains the platinum-based anticancer drug oxaliplatin, when they carried a variant in *ERCC6*, a major component of TCR. Platinum-based chemotherapy has been widely used in various cancers besides pancreatic cancer, and there have been several genetic studies related to platinum-based chemotherapy responses [28–30]. Among those studies, Cui et al. reported that *ERCC6* is associated with platinum-based chemotherapy responses in lung cancer patients [31]. The genetic variant of *ERCC6* reported in that study is also rs2228528, consistent with our study [31]. There were several previous studies investigating genes associated with platinum-based chemotherapy responses, including FOLFIRINOX in pancreatic cancer [11]. These previous studies reported that patients with mutations in the DDR gene showed a good response to FOLFIRINOX, but none of the genes associated with that response were related to the NER pathway [11,12]. Most previous studies were conducted using targeted gene sequencing that did not probe for *ERCC6*, so the gene in the NER pathway related to platinum-based chemotherapy response may not have been found [11,12,32]. However, by analyzing 148 DDR genes based on whole exome sequencing, we were able to find a novel genetic variant of *ERCC6* that is associated with FOLFIRINOX responses. There were no human data on chemotherapy agents related to *ERCC6* other than platinum chemotherapy agents, and there was one report showing that the anticancer effect of 5-fluorouracil was significantly increased when *ERCC6* was knocked down in colorectal cancer cell

lines and xenograft models [33]. Although the above study did not use pancreatic cancer cell, considering the inclusion of 5-fluorouracil in FOLFIRINOX regimen, it is likely that 5-fluorouracil in addition to oxaliplatin may have influenced the difference in response to FOLFIRINOX according to the *ERCC6* variant. This should be confirmed through future studies using pancreatic cancer cells.

To date, the most well-known DDR gene mutation related to treatment responsiveness to FOLFIRINOX in pancreatic cancer is the BRCA mutation [8]. The percentage of pathogenic BRCA mutations was as small as 1% in our study, and statistical significance may not have been found with respect to the response to FOLFIRINOX due to the small number of patients. Although there are differences according to reports, the prevalence of germline BRCA mutations in patients with pancreatic cancer is known to be highest at 10−14% in patients with Ashkenazi Jewish ancestry, and 4–7% in other Western patients [9,34]. In Asian patients, there are no reports based on a large cohort, but a study reported by Lee et al. showed a relatively low prevalence (0.6%) of germline BRCA mutations, which is consistent with our study [10]. Compared to the relatively low frequency of BRCA mutations, rs2228528 in *ERCC6* found in our study showed a high variant allele frequency (VAF) of 42.2%. According to large-scale reference genomic data, rs2228528 in *ERCC6* is a germline variant with a VAF of 18–24% globally and 41–47% in East Asia, showing similar frequency to our study [35–37]. Considering this high VAF, rs2228528 in *ERCC6* is a good candidate for a biomarker to predict FOLFIRINOX responses, and is expected to be an especially useful biomarker among Asian pancreatic cancer patients with low BRCA mutation frequency. Additionally, rs2228528 found in this study is a germline variant showing a VAF of around 40% and can be easily checked using blood samples. Therefore, if future studies validate

the association between rs2228528 in *ERCC6* and response to FOLFIRINOX, using blood samples, a single blood draw could provide an easy and fast way to determine whether to use FOLFIRINOX treatment or gemcitabine-based chemotherapy first in pancreatic cancer patients. If rs2228528 in *ERCC6* has a significant effect on protein expression, screening for this SNP using an immunohistochemistry-based technique may be possible, but this has not been revealed yet. In addition, since the cost of genetic testing has recently become cheaper and rs2228528 in *ERCC6* may be confirmed through a single blood draw, screening through this will be more accurate and efficient. Furthermore, poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors are also known to elicit a good response in pancreatic cancer patients with DDR gene mutations, so future studies are needed to determine whether *ERCC6* mutations play a role in the response to PARP inhibitors [38].

It is unclear whether rs2228528 in *ERCC6* directly affects the progression of pancreatic cancer regardless of chemotherapy. However, among DNA damage repair genes such as *ERCC6*, the well-known BRCA gene itself is considered to have no significant effect on the progression of pancreatic cancer, and it seems to show a difference in survival through a difference in responsiveness to platinum-based chemotherapy [39]. Similarly, it can be assumed that the rs2228528 in *ERCC6* itself does not affect the progression of pancreatic cancer.

This study had several strengths. First, this is the largest study to date on genes related to the responsiveness of FOLFIRINOX chemotherapy in patients with pancreatic cancer. Second, this study discovered novel *ERCC6* variants by conducting genetic analysis based on whole exome sequencing rather than using the targeted gene sequencing methods from previous studies.

There are some limitations in this study. First, this study was conducted in a single institution and analyzed in a retrospective manner, although a prospective database was used. This retrospective nature may have caused selection bias. In particular, in order to include only patients whose PFS was measured more accurately, we excluded patients with follow-up loss or patients who could not continue chemotherapy due to poor performance. As a result, selection bias may have occurred in the direction of including patients with better performance status. However, it would be difficult for this direction of selection bias to work solely to make the insignificant *ERCC6* variant appear significantly, and rather, it is meaningful that even in patients with good performance, the responsiveness to FOLFIRINOX was different according to the *ERCC6* variant. Second, there was no validation process in this study. Whether rs2228528 alters protein structure has yet to be confirmed, so further research is needed on this, and validation studies in large-scale patients are required. Third, there was no matched blood sample that could identify the germline variant, so the distinction between germline and somatic variants was not made. However, this distinction could be roughly made with existing large-scale genomic data, and in the case of DDR gene mutations, both germline and somatic mutations are known to have an effect on the response to platinum-based chemotherapy. Therefore, this distinction of germline and somatic mutation should not have a critical effect on the results of this study [11,40]. Fourth, pancreatic cancer patients with the A alleles of rs2228528 in ERCC6 showed improvement in PFS but not significantly better response rates. In this regard, future large-scale studies are needed. However, although the rs2228528 genotype did not show a significant association with response rate, a significant association with improved PFS and disease control rates could help determine

whether to use FOLFIRINOX chemotherapy as the first-line.

Chapter 5. Conclusions

In conclusion, we found a novel variant in *ERCC6* associated with improved PFS in pancreatic cancer patients who underwent FOLFIRINOX chemotherapy. If validated through future large-scale studies, rs2228528 in *ERCC6* could be used as a valuable biomarker to help determine whether to use FOLFIRINOX as the first-line therapy in pancreatic cancer patients.

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Table 1. Detailed list of DNA damage repair genes.

DNA damage repair pathway	Genes
Base excision repair	APEX1, APEX2, APLF, DUT, LIG3, MBD4, MPG, MUTYH, NEIL1, NEIL2, NEIL3, NTHL1, NUDT1, OGG1, PARP1, PARP2, PNKP, POLB, RECQL4, SMUG1, TDG, TDP1, UNG, WRN, XRCC1
Direct reversal repair	ALKBH2, ALKBH3, MGMT
DNA damage response	ATM, ATR, ATRIP, CHEK1, CHEK2, CLK2, CLSPN, H2AFX, HUS1, MDC1, NABP2, PER1, PER2, RAD1, RAD17, RAD9A, RNF8, TOP2A, TOPBP1, TP53, TP53BP1, UBE2N
Fanconi Anemia	BRCA2, BRIP1, FAAP100, FAAP24, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, PALB2, RAD51C, SLX4, USP1, WDR48
Homologous recombination	BARD1, BLM, BRCA1, DMC1, EME1, EME2, GEN1, HELQ, MRE11A, MUS81, NBN, RAD50, RAD51, RAD51B, RAD51D, RAD52, RAD54B, RAD54L, RBBP8, RECQL, RECQL5, SHFM1, SLX1A, SLX1B, XRCC2, XRCC3
Mismatch repair	MLH1, MLH3, MSH2, MSH3, MSH4, MSH5, MSH6, PCNA, PMS1, PMS2, POLD1, POLE
Non-homologous end joining	DCLRE1C, LIG4, NHEJ1, POLL, POLM, PRKDC, PRPF19, SETMAR, XRCC4, XRCC5, XRCC6
Nucleotide excision repair	CCNH, CDK7, CETN2, DDB1, DDB2, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC8, GTF2H1, GTF2H2, GTF2H3, GTF2H4, GTF2H5, LIG1, MMS19, MNAT1, RAD23A, RAD23B, RPA1, RPA2, RPA3, RPA4, UVSSA, XAB2, XPA, XPC

Table 2. Patient characteristics.

Variables	Median (IQR) or Number (%)
Age, years	64.0 (58.0–70.0)
Sex	
Male	58 (56.3)
Female	45 (43.7)
Body mass index, kg/m^2	22.9 (21.1–25.1)
ECOG performance status	
0	99 (96.1)
1	4 (3.9)
Tumor location	
Head	43 (41.7)
Body/tail	60 (58.3)
Clinical T stage	
T1–3	38 (36.9)
T4	65 (63.1)
Clinical N stage	
N0	73 (70.9)
N1–2	30 (29.1)
Clinical M stage	
M0	66 (64.1)
M1	37 (35.9)
Serum CA 19-9, U/mL	675.0 (63.0–4492.0)
Best response to FOLFIRINOX chemotherapy	
Partial response	33 (32.0)
Stable disease	57 (55.3)
Progressive disease	13 (12.6)
Resection after FOLFIRINOX chemotherapy	41 (39.8)

Abbreviations: IQR, interquartile range; ECOG, Eastern Cooperative Oncology Group; CA 19-9, carbohydrate antigen 19-9.

Table 3. Summary of FOLFIRINOX treatment according to rs2228528 in *ERCC6*

Variables	Genotype of rs2228528 in *ERCC6*			*p*
	GG genotype (n = 39)	GA genotype (n = 41)	AA genotype (n = 23)	
Response – no. (%)				
Complete response	0	0	0	
Partial response	10 (25.6)	16 (39.0)	7 (30.4)	
Stable disease	20 (51.3)	21 (51.2)	16 (69.6)	
Progressive disease	9 (23.1)	4 (9.8)	0	
Response rate – no. (%)	10 (25.6)	15 (36.6)	7 (30.4)	0.570
Disease control rate – no. (%)	30 (76.9)	37 (90.2)	23 (100.0)	0.024
Surgical resection rate after FOLFIRINOX treatment – no. (%)	10 (25.6)	19 (46.3)	12 (52.2)	0.065

Table 4. Multivariate Cox proportional hazard regression of factors associated with progression-free survival (PFS).

Variables	**HR (95% CI)**	***p***
ERCC6 genotype	0.539 (0.371–0.783)	0.001
GG	reference	
GA		
AA		
Age	1.032 (0.998–1.066)	0.062
Sex	0.757 (0.449–1.275)	0.295
Male	reference	
Female		
T stage	0.691 (0.382–1.251)	0.223
T1–3	reference	
T4		
N Stage	1.574 (0.893–2.774)	0.116
N0	reference	
N1–2		
M Stage	2.209 (1.159–4.209)	0.016
M0	reference	
M1		
Tumor location	0.578 (0.311–1.075)	0.083
Head	reference	
Body/tail		
Body mass index	0.860 (0.500–1.481)	0.587
≤23	reference	
>23		
CA 19-9	0.915 (0.491–1.706)	0.780
≤37	reference	
>37		
Surgical resection after FOLFIRINOX	0.265 (0.135–0.520)	<0.001
No	reference	
Yes		

Abbreviations: HR, hazard ratio; CI, confidence interval; CA 19-9, carbohydrate antigen 19-9.

Table 5. Cox regression analysis for evaluating the association between progression-free survival and three variants derived from synthetic association analysis of rs2228528.

Gene - Variants	HR (95% CI)	*p*	FDR adjusted *p*
AIFM2 – rs10999147	0.783 (0.416–1.473)	0.448	0.905
CASP7 – rs11555408	1.194 (0.639–2.229)	0.579	0.934
JMJD1C – rs10761725	0.437 (0.253–0.753)	0.003	0.411

Abbreviations: HR, hazard ratio; CI, confidence interval; FDR, false discovery rate.

Figure 1. Flow chart of patient enrollment. The flowchart shows the patient exclusion criteria and the final number of patients included in the study.

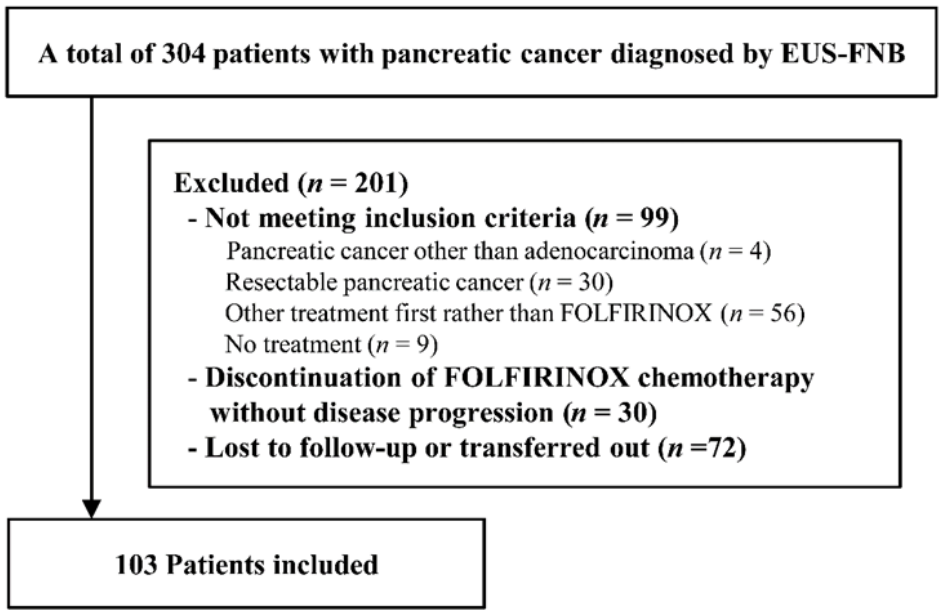


Figure 2. Schematic diagram of genetic variant analysis. The schematic diagram shows how genetic variants were selected and analyzed from whole exome sequencing data. Abbreviation: WXS, whole exome sequencing.

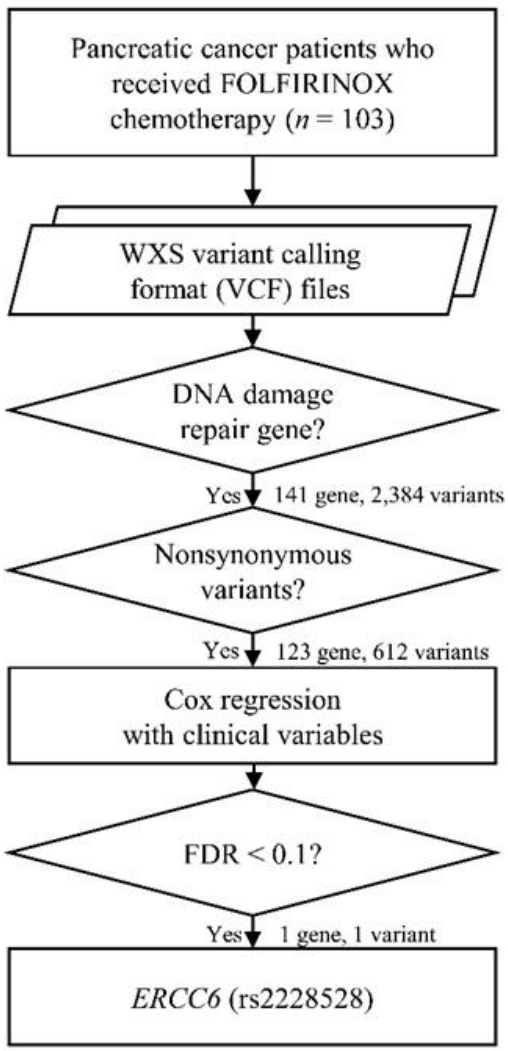
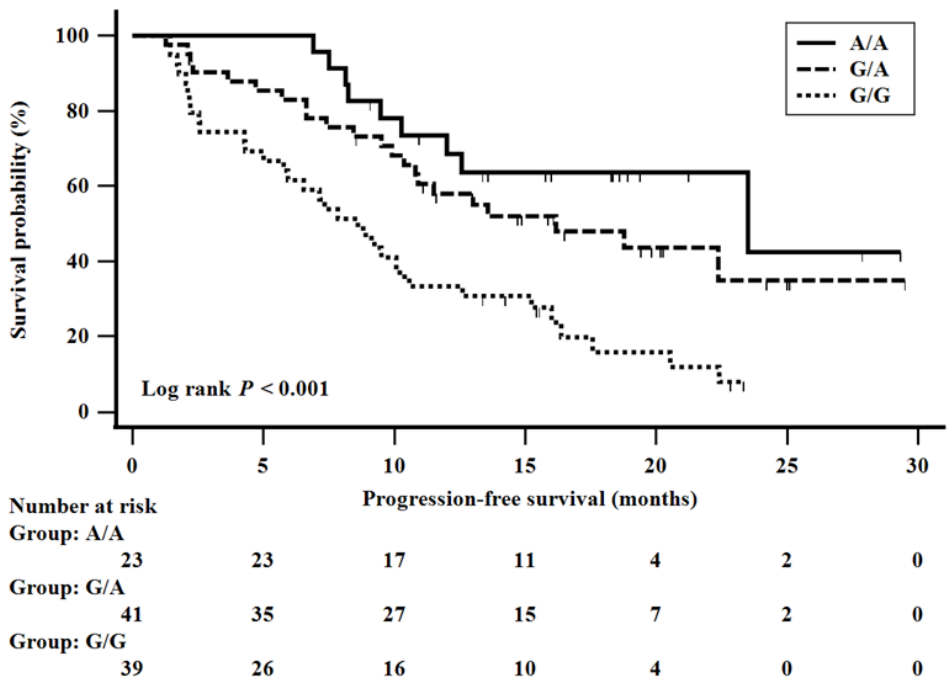


Figure 3. Progression-free survival according to genotype of rs2228528 in ERCC6. Patients were divided according to rs2228528 genotypes: AA (n = 23), GA (n = 41), and GG (n = 39), and assessed for progression-free survival using the Kaplan–Meier method and the log-rank test.



Number at risk							
Group: A/A							
	23	23	17	11	4	2	0
Group: G/A							
	41	35	27	15	7	2	0
Group: G/G							
	39	26	16	10	4	0	0

Figure 4. Flow chart of RNA expression analysis according to genotype of rs2228528 in *ERCC6*. The flow chart shows how expression analysis was performed using data from the 1000 Genomes Project.

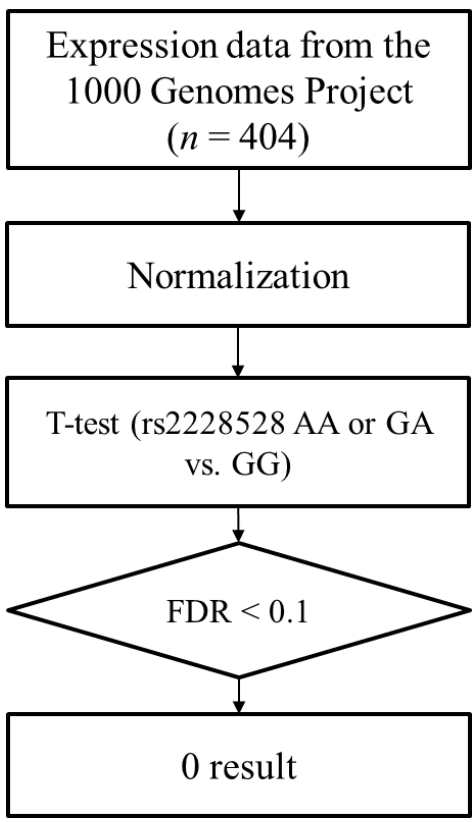
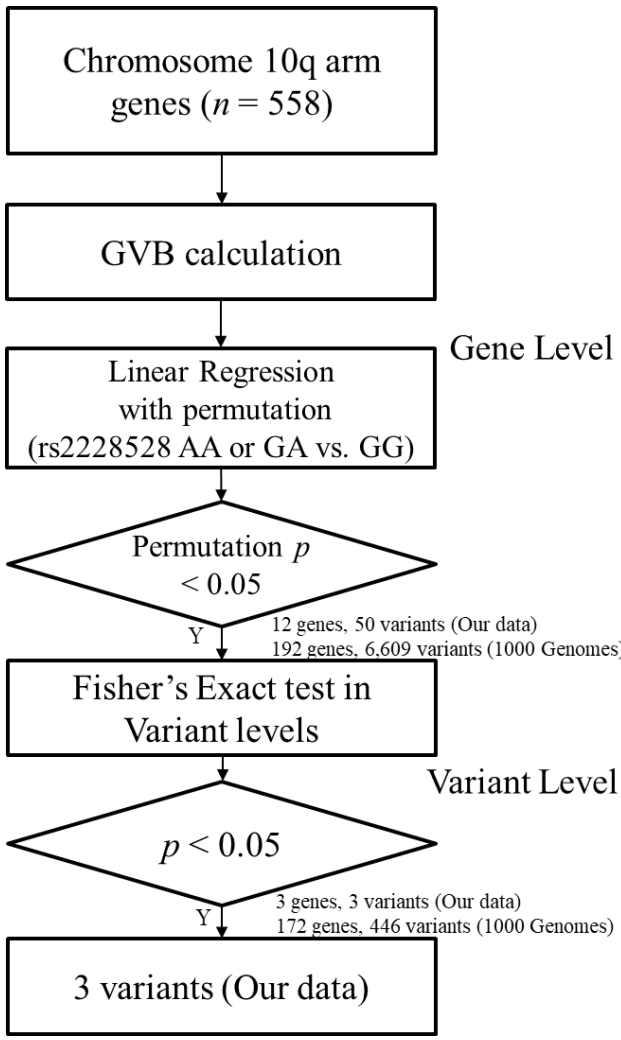


Figure 5. Flow chart of synthetic association analysis. The flow chart shows the process of synthetic association analysis starting with GVB calculations.



국문 초록

FOLFIRINOX는 현재 췌장암 환자의 표준 항암화학요법 중의 하나로, DNA 손상 복구 유전자 중 하나인 BRCA 유전자에 변이가 있는 췌장암 환자에서 더 효과적인 것으로 알려져 있다. 그러나 BRCA 유전자 변이는 췌장암 환자, 특히 아시아계 환자에서는 드물다. 이에 본 연구를 통해 췌장암 환자의 FOLFIRINOX 항암화학요법에 대한 반응과 관련된 새로운 DNA 손상 복구 유전자 변이를 발견하고자 하였다. 연구대상은 FOLFIRINOX 항암화학요법을 첫 번째 치료로 받았으며, 내시경 초음파 유도 생검을 통해 채취한 조직이 DNA 염기 서열 분석에 적합했던 췌장암 환자 코호트를 이용했다. 전장엑솜염기서열분석을 기반으로 148 개의 DNA 손상 복구 유전자 변이를 탐색하고, 다변량 Cox 회귀 분석을 시행하여 무진행생존기간과 관련된 유전자 변이를 찾고자 하였다. 총 103 명의 환자가 연구에 포함되었으며, 141 개의 DNA 손상 복구 유전자의 2,384 개 변이들 중 123 개 유전자에서 발견된 612 개의 비동의 (non-synonymous) 변이가 Cox 회귀 분석을 위해 선택되었다. 다변량 Cox 모델에서 *ERCC6* 유전자의 rs2228528이 개선된 무진행생존기간 (위험비, 0.54, p = 0.001) 과 유의하게 연관되어 있었다. 무진행생존기간 중앙값은 rs2228528 유전자형 AA인 환자 (23.5 개월)가 유전자형 GA (16.2 개월) 및 GG (8.6 개월)인 환자보다 유의하게 길었다 (로그 순위 p < 0.001). 이 연구는 *ERCC6*의 rs2228528이 췌장암 환자의 FOLFIRINOX 항암화학요법

에 대한 반응의 잠재적인 예측인자가 될 수 있음을 시사한다.

주요어 : 췌장암; FOLFIRINOX; DNA 복구; *ERCC6*; 무진행생존기간

학번 : 2017-39638