CANCER RESEARCH AND TREATMENT (CRT)

Original Article

Clinical Impact of Somatic Variants in Homologous Recombination Repair-Related Genes in Ovarian High-Grade Serous Carcinoma

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Abstract

Purpose

In this study, we investigated the frequencies of mutations in DNA damage repair genes including *BRCA1*, *BRCA2*, homologous recombination genes and *TP53* gene in ovarian high-grade serous carcinoma, alongside those of germline and somatic *BRCA* mutations, with the aim of improving the identification of patients suitable for treatment with poly(ADP-ribose) polymerase inhibitors.

Materials and Methods

Tissue samples from 77 Korean patients with ovarian high-grade serous carcinoma were subjected to next-generation sequencing. Pathogenic alterations of 38 DNA damage repair genes and *TP53* gene and their relationships with patient survival were examined. Additionally, we analyzed *BRCA* germline variants in blood samples from 47 of the patients for comparison.

Results

BRCA1, *BRCA2*, and *TP53* mutations were detected in 28.6%, 5.2%, and 80.5% of the 77 patients, respectively. Alterations in *RAD50*, *ATR*, *MSH6*, *MSH2*, and *FANCA* were also identified. At least one mutation in a DNA damage repair gene was detected in 40.3% of patients (31/77). Germline and somatic *BRCA* mutations were found in 20 of 47 patients (42.6%), and four patients had only somatic mutations without germline mutations (8.5%, 4/47). Patients with DNA damage repair gene alterations with or without *TP53* mutation, exhibited better disease-free survival than those with *TP53* mutation alone.

Conclusion

DNA damage repair genes were mutated in 40.3% of patients with high-grade serous carcinoma, with somatic *BRCA* mutations in the absence of germline mutation in 8.5%. Somatic variant examination, along with germline testing of DNA damage repair genes, has potential to detect

additional candidates for PARP inhibitor treatment.

Key words

Epithelial Ovarian Carcinomas, Homologous Recombination Repair, Massively Parallel Sequencing

Introduction

Ovarian cancer is the leading cause of death among patients with gynecological malignancies worldwide, and approximately 225,000 ovarian cancers are newly diagnosed each year. The lack of an effective screening test and unfavorable anatomy are associated with advanced-stage disease at diagnosis and poor prognosis for the majority of patients. Overall survival is poor among gynecologic cancers, with a 5-year survival rate of 44% for all stages and 140,000 related deaths occurring annually worldwide, the majority of which are from high-grade serous carcinomas (HGSCs) [1-3].

Current estimates indicate that 20%-25% of women have an inherited germline mutation predisposing them to ovarian cancer [2]. In approximately 65%-85% of hereditary ovarian cancers, the associated genetic abnormality is a germline mutation of one of the *BRCA* genes (*BRCA1* or *BRCA2*). Several other tumor suppressor genes and oncogenes are also associated with hereditary ovarian cancer, including homologous recombination (HR) DNA repair genes. The Cancer Genome Atlas (TCGA) reported HR deficiency in approximately 50% of patients with high-grade serous ovarian cancer [4]; however, in TCGA study, the majority of patients were European, with the percentage of Asian patients being only 3.2% (19/316).

The identification of a mutation in an ovarian cancer susceptibility gene represents a fundamental step in the diagnosis and treatment of these tumors. Moreover, the identification of a mutation in patients who have already been diagnosed can provide information about the pathogenesis of their tumors. With the development of targeted therapy, current strategies for the control and prevention of ovarian cancer rely on a thorough understanding of contributing genetic factors, at both the germline and somatic levels. In this context, next-generation sequencing technologies provide an unprecedented opportunity to simultaneously analyze multiple cancer susceptibility genes, reduce delays and costs, and optimize the molecular

diagnosis of hereditary ovarian cancer.

Poly (ADP-ribose) polymerase (PARP) inhibitors have shown clinical effects in patients with ovarian cancer showing *BRCA* dysfunction or homologous recombination deficiency (HRD). PARP inhibitors were originally designed for synthetic lethal interaction with *BRCA1* or *BRCA2* mutations [5, 6]. *In vitro* studies have demonstrated that defects in the other HR proteins, such as ATM, CHEK1, CHEK2, NBN, and RAD51D, also confer sensitivity to PARP inhibitors [7, 8]. Further, PARP inhibitors are active in a subset of sporadic (*BRCA* wild-type) recurrent platinum-sensitive ovarian carcinomas [9], which may be attributable to the influence of undetected HR gene alterations in that study. The possible application of PARP inhibitors as a therapeutic option for patients with ovarian cancer, and alterations in genes other than the *BRCA* genes, is currently under investigation (NCT02476968, ORZORA study).

TP53 mutation is found in many cancer types and is related to DNA damage response and apoptosis [10]. It is well known that *TP53* mutations are associated with poor prognosis in several cancers including ovarian cancers [10, 11]. However, the relationship between DNA damage repair (DDR) gene and *TP53* gene alterations and their combined effect on HGSC patient outcome has not been well described.

In this study, we investigated variants in DDR genes and *TP53* gene in Korean patients with HGSC, analyzed their frequency and characteristics in relation to germline and somatic *BRCA* mutations in this group, and analyzed their impact on clinical outcome to provide better prediction for PARP inhibitor therapy response.

Materials and Methods

1. Patients and specimens

Eligibility criteria were as follows: women aged 20 years or older with pathological

diagnosis of epithelial ovarian, fallopian tube, or peritoneal carcinoma, with a high-grade serous histologic component. Patients were treated using standard treatments (cyto-reductive surgery and/or platinum-based chemotherapy) at the time of diagnosis.

Family history of cancer was recorded and confirmed by direct contact with the patients and their families. A patient was considered to have a family history of cancer if any of the following criteria were met: (1) if there were one or more cases of ovarian, peritoneal, fallopian tube, breast, pancreas, or prostate cancer among first- or second-degree relatives; or (2) if the patient had a history of primary breast cancer.

Fresh frozen or formalin-fixed paraffin-embedded (FFPE) tumor tissue samples from the 77 patients with HGSC were analyzed. Among these 77 patients, blood samples were available from 47 patients for *BRCA* germline variant analysis. Fifty-nine cases with fresh tumor tissue, 48 available matched normal (pair in the same case) FFPE tissue for whole exome sequencing (diagnosed between the year 2005 and 2014), and 18 cases of FFPE tumor tissue for panel sequencing (diagnosed between 2017 and 2018) were obtained from the archive of Department of Pathology, CHA Bundang Medical Center. Two pathologists (H.K. and S.K.) reviewed the histology (Fig. 1) using the 2014 World Health Organization classification criteria [1].

2. DNA sequencing and bioinformatics analysis of pathogenic variants

Genomic DNA was extracted from a single surgical sample, containing a HGSC component, from each patient. Two different targeted sequencing assays were carried out: (1) whole exome sequencing and (2) targeted gene panel sequencing.

Fifty-nine fresh tumor samples for whole exome sequencing were snap frozen, and genomic DNA was extracted using GeneAll Exgene^{TM} Clinic SV Kit columns (GeneAll, Cambio, United Kingdom), according to the manufacturer's protocols. Exomes were captured

using the Agilent SureSelect Exome V5 probe set, and the captured DNA was sequenced on the Illumina HiSeq 2500 platform. Sequences were aligned using BWA-0.7.15, based on Genome Reference Consortium build 37 (GRCh37). Duplicated reads were removed using Picard-tools 2.7.1, and variants were called, according to GATK 3.8 best practices (https://software.broadinstitute.org/gatk/best-practices/). We performed whole exome sequencing with the matched normal FFPE lymph node tissue of 48 patients among the 59 patients, available matched normal tissue was present.

For 18 FFPE tumor tissue samples for panel sequencing, genomic DNA was extracted using the RecoverAll multi-sample RNA/DNA isolation workflow (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocols. One hundred forty-three genes were targeted using the Ion Torrent Oncomine Comprehensive Assay v1 (OCA1, Thermo Fisher Scientific, Waltham, USA) and sequenced using an Ion S5 XL. Sequences were aligned using Torrent Suite 5.10.0, based on GRCh37, and variants were called with Torrent Variant Caller 5.10-9.

Thirty-eight DDR genes (including *BRCA 1/2*, HR genes, mismatch repair genes) and *TP53* gene were selected for analysis of the pathogenic alterations with fresh or FFPE tissues in 77 patients.

Among them, for comparison of germline and somatic *BRCA1* and *BRCA2* variants of twenty-nine whole exome sequencing patients and 18 panel sequencing patients were analyzed. Germline Sanger sequencing was separately conducted with blood samples in 47 patients among 77 patients (Tables 1 and 2).

For identifying pathogenic variants from germline common variants and reducing false-positive variants without available matched normal samples, we took three strategies of filtering out common germline or false-positive variants. Firstly, we removed variants with low qualities (QUAL score < 30 or depth < 20 or FILTER=FAIL). Secondly, we removed common variants

(allele frequency > 0.01) in dbSNP database (00-common_all.vcf file was downloaded on June 7, 2018) or in ExAC database (ExAC.r1.sites.vep.vcf file was downloaded on July 16, 2018). Lastly, we removed common variants in Korean (allele frequency > 0.01) based on KOVA (K1055E_allele_frequency.txt was downloaded on November 15, 2017) and KRGDB (variants1100_cmm.txt file was downloaded on December 1, 2016 from http://coda.nih.go.kr/coda/KRGDB).

Fresh tumor somatic variant calls and the matched normal germline variant calls for 48 whole exome sequencing patients were carried out using the same GATK 3.8 best practice pipeline and GATK4.1-Mutect2 Tumor-Normal pair pipeline (FILTER=PASS and Depth ≥ 20), respectively. The information of germline or somatic variants is provided in S1 and S2 Tables.

3. Annotation and classification of pathogenic mutations

For functional annotation of each variant, ANNOVAR [12] , Variant Effect Predictor [13], and Ion Reporter ver. 5.2 software (Thermo Fisher Scientific, Waltham, USA) was used. For interpretation of the pathogenicity of each variant, ClinVar, Oncomine Knowledgebase, and guidelines for the interpretation of sequence variants in cancer (American College of Medical Genetics) were referred to [14]. Among the whole exome sequencing and panel sequencing test results, information on 38 DDR genes and *TP53* gene was obtained (S1-S3 Tables). These 39 genes were selected for inclusion for a more focused analysis on *BRCA* and HRD genes. To assess the clinical impact of these genes, two subgroups according to the mutation status of *TP53* and the other 38 DDR genes of patients were separately analyzed for their survival. The groups were defined as follows: group 1 includes patients with pathogenic mutation in any of the 38 DDR genes, regardless of *TP53* mutation (n=31), and group 2 includes patients with *TP53* mutation only (n=37).

4. *BRCA* germline variant analysis

Of the 77 patients who underwent somatic genetic analysis, 47 had previously undergone germline *BRCA* testing, and genetic information on germline *BRCA* status was obtained and compared with the somatic *BRCA* test results. All germline tests were carried out using the Sanger sequencing method, as previously described [15] .

5. Statistical analysis

To evaluate the differences in overall survival and disease-free survival of the group 1 (any DDR gene mutation–positive regardless of *TP53* mutation status) and group 2 (*TP53* mutation only) were statistically significant, the log-rank test was used. Survival analysis was conducted using the survival and survminer R packages and the p-value of each Kaplan Meier-plot was calculated by log-rank test. Survival plots were depicted in ggplot2 R package.5.

6. Ethical statement

Women who met the criteria (n=77) provided written informed consent between 2009 and 2018, and the study was conducted with approval from the Institutional Review Board of the CHA Bundang Medical Center (IRB approval No. 2016-10-010-005). Use of patient-derived samples for this study was approved, and the work described was performed in accordance with approved guidelines.

Results

1. Patient clinical characteristics

The clinical characteristics of the 77 patients included in the study are listed in Table 3. The majority ethnicity was Korean, with only four individuals of Korean Chinese ethnicity. Median

age at diagnosis was 56 years (range, 36 to 82 years). Primary cancers were ovary (92.2%), fallopian tube (3.9%), and peritoneum (3.9%). Histologically, the majority of tumors were HGSC. One of two patients with carcinosarcoma had HGSC in the left ovary and metastatic lesions and carcinosarcoma in only the right ovary. In this patient, only the HGSC component was used for tumor genetic testing. Approximately 90% of patients had advanced-stage disease (> stage III). Six patients (7.8%) were previously diagnosed with breast cancer; 17 (22.1%) had a family history of breast, ovarian, fallopian tube, peritoneal, pancreas, or prostate cancer in first- or second-degree relatives. Twenty-one patients (21/77, 27.3%) had positive "family history," according to the definition used in the present study.

2. Pathogenic and likely pathogenic variants in the 38 analyzed DDR genes and *TP53* gene in tissue samples

One or more mutation in the DDR genes was identified in 40.3% (31 of 77) of patients. Of the 38 DDR genes and *TP53* gene, pathogenic mutations were identified in eight genes; the remaining 31 genes were wild type. Specific details of the 98 pathogenic mutations and 63 variants of unknown significance identified in these 39 genes in the 77 patients are listed in S1-S3 Tables. Observed pathogenic and likely pathogenic mutations are shown in Fig. 2. *BRCA1* (28.6%), *BRCA2* (5.2%), and *TP53* (80.5%) were the most frequently altered genes. *BRCA* and *TP53* mutations were detected pervasively. The frequencies of the detected variants are shown in S4 Fig. Among *BRCA* gene mutations, *BRCA1* Y130Ter variants were observed in three patients (13.6%, 3/22). The *TP53* alterations observed with frequency of more than two patients are as follows. *TP53* R175H (5.6%) and *TP53* R248Q/W (7.9%) were observed in three (4.8%) and two (3.2%) patients among 62 patients, respectively. *TP53* K132R, I195T, Y220C, and R306* variants were observed in three patients (4.8%). *TP53* mutations were significantly

enriched in DNA-binding domain (binomial test p-value=1.38e-4). The details of the *TP53* gene mutation in this study is shown in graphic chart in S5 Fig.

DDR gene alterations other than *BRCA1/2* genes were detected in (in order of frequency) *RAD50* (5.1%), *ATR* (3.4%), *MSH2* (2.6%), *FANCA* (1.7%), and *MSH6* (1.7%). One patient (Pat53) carried four mutations (*TP53*, *RAD50*, *ATR*, and *MSH2*) simultaneously, while another two patients each had three different mutations.

To assess the clinical impact of these genes, two subgroups of patients were analyzed for their survival (Fig. 3A), according to the mutation status of 38 DDR genes and *TP53* gene as follows: group 1 (pathogenic mutation in any of the 38 DDR genes, regardless of *TP53* mutation); group 2 (*TP53* mutation only). The overall survival and disease-free survival of patients in these two groups did not differ significantly (p=0.12) (Fig. 3B); however, disease-free survival was different (p=0.05) (Fig. 3C). The disease-free survival of patients with at least one pathogenic mutation in any of the 38 DDR genes was better than that of patients with *TP53* mutations only.

Nine patients were interpreted negative for DDR genes and *TP53* gene mutation. They were not included for survival analysis because the number of patients was too small for meaningful statistical analysis.

3. Comparison between germline and somatic *BRCA* mutations

Pathogenic variants of *BRCA* genes were detected in 20 of the 47 patients (42.6%) in total: 17 in *BRCA1* and 3 in *BRCA2*. Among these, germline variants were identified in 16 (34.0%) of the 47 patients; 14 of the variants were in *BRCA1*, with two in *BRCA2*. Of note, four patients (8.5%) had only somatic *BRCA* variations without germline mutation (Table 4).

Discussion

The most common histologic type of ovarian cancer is epithelial ovarian carcinoma (EOC), which has five subtypes: high-grade serous (70%), endometrioid (10%), clear cell (10%), low-grade serous (5%), and mucinous (3%). EOC is both clinically diverse and molecularly heterogeneous, and its subtypes have distinct gene expression patterns. Ovarian HGSC is distinct from non-HGSC, differing with respect to clinical presentation, disease distribution, response to therapy, survival, and site of origin [16]. Pennington et al. reported that non-HGSC cases had some *BRCA* mutations and a greater proportion of mutations in other HR genes [17]. Therefore, we focused on tumors with HGSC histology and excluded non-HGSC tumors from the present study.

The frequency of somatic *TP53* mutations in Korean patients with HGSC in the present study was lower (80.5%) than expected. High-grade serous ovarian carcinoma is characterized by ubiquitous *TP53* abnormalities, with *BRCA* changes in approximately 50% of cases, along with chromosomal instability. *TP53* aberration has been considered a driver event in ovarian carcinogenesis, and it is possible that *TP53* mutations are more stable over time than other mutations. *TP53* was mutated in 95.9% (303 of 316) of HGSC samples in TCGA 2011 study [4]; however, in the present study, the frequency of *TP53* mutation among HGSC patients was 80.5%. When we investigated the frequency of *TP53* mutations in 10 Asian patients included in TCGA 2011 study, nine (90%) of them had *TP53* mutations. There was no statistically significant association between incidence of *TP53* mutations and ethnicity (Asian vs. others [mostly European]) in TCGA data set (p=0.35). Regarding the quality of sequencing, OCA1 panel that we used in this study includes the whole exon regions of *TP53* as well as whole exome sequencing, and the average coverage of all targeted regions of *TP53* in two sequencing methods were 98X for whole exome sample, and 3576X for OCA1 panel sample. We provided

the sequencing coverage of overall targets and of *TP53* in each patient in S3 Table. We also confirmed that there was no statistical difference of sequencing coverage between patients with *TP53* mutation versus without *TP53* mutations. It is shown in S6 Fig. There was no statistical difference of sequencing coverage between patients with *TP53* mutations versus without *TP53* mutations, even though the average coverage values in two sequencing methods are different (S6 Fig.). However, when we investigated the *TP53* variants with low quality (depth < 20), there were *TP53* mutations in seven patients. If we included seven *TP53* mutations with low quality, the frequency of *TP53* mutations would be 89.6%, comparable to TCGA 2011 study. The information on mutations with low quality (depth < 20) are provided in S7 Table.

We compared the disease-free survival of the subset of HGSCs with DDR gene mutation with or without *TP53* mutation (group 1) and *TP53* mutations only (group 2) to examine the effect of these alterations on disease-free survival of the HGSC patients. The disease-free survival of group 1 was better than that of group 2 (Fig. 3C), which is in accordance with previous studies that *BRCA1*, *BRCA2* gene mutation and HRD are related to favorable clinical outcome [18, 19]. *TP53* mutation status and its impact on survival is different between low grade and HGSCs, but among the HGSCs, *BRCA* and HR genes have more impact on survival than *TP53* mutation status.

When DNA damage repair genes are affected, the cancer cells may become more sensitive to anticancer agents directed to DNA damage such as platinum-based antineoplastic therapy. Because many anticancer therapy agents are directed to DNA damage such as alkylating agents. If the DNA damage increases without repair, it may lead to cell death. Such tumors may also produce more neoantigens due to increased mutational load. It may also elicit more immune responses to tumor and these patients may also be the candidates for cancer immunotherapy [11].

Estimates of the contribution of germline *BRCA* mutations to EOC vary widely, from 5% to 20% [20], and patients having somatic mutations without germline mutations in *BRCA* are less frequent, accounting for 2%-8% [21-24]. Germline and somatic *BRCA* mutation frequencies in EOC patients, according to the published literature, are listed in Table 5 [4, 23-27]. As for HGSC, the lowest frequency of germline and somatic *BRCA* mutations (22.5%) is recorded in TCGA 2011 data, with the highest (20/46, 43.5%) in the present study. The mean ratio of HGSC patients with only somatic *BRCA* mutations to both germline and somatic mutations in the literature is 25.2% (range, 16.1% to 33.3%). Germline *BRCA* testing is widespread in recent clinical practice; however, this approach could miss the patients with only somatic *BRCA* mutations, who could also potentially benefit from therapy with PARP inhibitors.

Although previous reports on the effectiveness of PARP inhibitors in cancers with *BRCA* and HR repair defects have mainly focused on germline alteration of these genes, such treatment may also be effective for patients with somatic aberrations. Some patients with ovarian cancer who do not carry germline *BRCA* mutations also respond to PARP inhibitors [28], suggesting that broader dysfunction of genes, other than the *BRCA* genes. The patients with DDR mutation other than BRCA mutation may also be responsive to PARP inhibitors. The high frequency of HRD in HGSC in this study suggests that screening for this phenotype should be considered to identify candidates for PARP inhibitor treatment among these patients.

In the present study, HGSC showed somewhat higher germline and/or somatic *BRCA* mutation frequency in Korea (20/47 cases, 42.6%), compared with the results of previous studies (22.5%-30.1%) (Table 5). When we reviewed our germline and somatic genetic test results, and there were no false-positive results or variants of unknown significance. In the present study, the germline *BRCA* mutation rate (16/47 cases, 34.0%) was higher than previous reports in other countries (16.1%-25.2%), but the somatic *BRCA* mutation rate (4/47 cases,

8.5%) did not show much difference with the previous reports (4.9%-8.7%). It was reported that germline *BRCA* mutation frequency of Korean epithelial ovarian cancer patients was higher than previous reports [15]. In another recent study of ovarian advanced high-grade serous carcinoma in Korean patients, germline *BRCA* mutation was found in 39.8% (51/128 cases), which is similar to the present study [29]. The possible reasons for higher rate of germline *BRCA* mutation in this study may be due to ethnic variation. Another reasons for differences in germline *BRCA* mutation rate maybe inclusion of primary fallopian tube or primary peritoneal HGSC cases which seem to show higher rate of *BRCA* mutation rate than primary ovarian cancer, or heterogeneity of cases included in different studies. Whether the study includes only HGSC or other epithelial ovarian cancer, inclusion of fallopian tube or primary peritoneal cancer, proportion of patients with family history may influence the detection rate of germline *BRCA* mutation.

We did not perform multiplex ligation-dependent probe amplification (MLPA) test to detect large genomic rearrangements (LGRs) in this study. Prevalence of LGRs was reported to be 1.8% in Korea at recent study[30]. The frequency of BRCA mutation would have been slightly higher if the MLPA test had been performed.

The reported prevalence of germline *BRCA* mutations in patients with fallopian tube or peritoneal cancers ranges from 15.8% to 40.9% [31]; however, *BRCA* mutations (two germline and two somatic) were found in four of five patients (80.0%) with fallopian tube or peritoneal cancers in the present study (Table 4). The one remaining patient (Pat17) with fallopian tube cancer had somatic *TP53* and *FANCA* mutations (S3 Table). Although a small number of subjects with peritoneal and fallopian tube cancers were included in this study, these cancers exhibited higher frequency of *BRCA* mutation than ovarian cancers, and all of the cases had at least one alteration among the 39 genes included in this study. These data require confirmation

by further study using a larger number of cases; however, it may imply that more candidates for PARP inhibitor therapy may be detected among patients with fallopian tube or peritoneal HGSC.

In conclusion, in this study we examined variants in DDR genes and *TP53* gene in patients with ovarian HGSC. To the best of our knowledge, this study is the first to analyze somatic *BRCA1*, *2* and DDR gene mutations and compare with germline and somatic *BRCA* mutations in Korean patients with HGSC. Mutations of DDR genes were observed in 40.3% (31/77), including *BRCA* mutations, and the frequency of *TP53* mutation (80.5%) was low compared with that in previous reports. Patients carrying somatic *BRCA* mutations without germline mutations were identified at a frequency of 8.5%. Although further validation in a large-scale study is needed, our data strongly indicate that more candidates for PARP inhibitor treatment can be detected by examination of somatic *BRCA* gene variants and other DDR genes, alongside germline testing for *BRCA* genes.

Conflict of Interest

Min Chul Choi received research funding from Chong Kun Dang Pharmaceutical Corp., Korea. All remaining authors have declared no conflicts of interest.

Acknowledgments

This work was supported by a grant (HI16C1559) from the Korea Health Technology R&D Project through The Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea. We thank the investigators and study teams. Park SI, a research nurse, supported clinical data collection and analysis.

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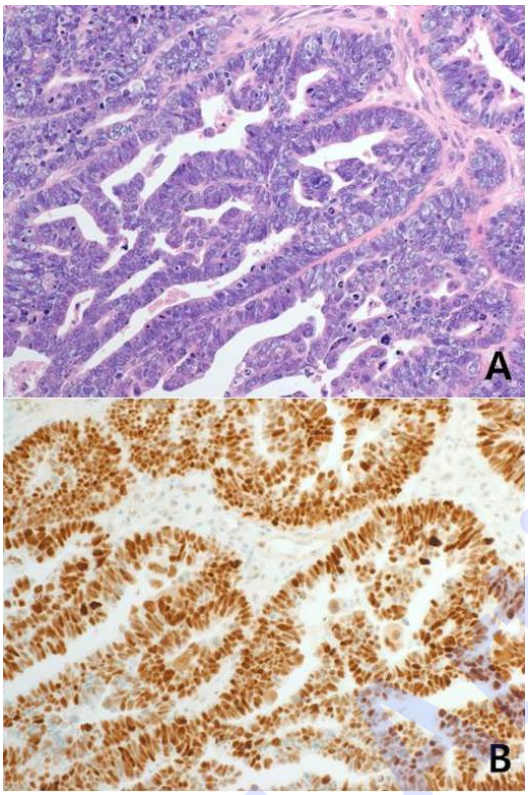


Fig. 1. Histologic features of high-grade ovarian serous carcinoma. (A) High-grade serous carcinoma (HGSC) composed of papillary, glandular patterns with large, hyperchromatic, pleomorphic nuclei and numerous mitoses is shown (H&E staining, ×200). (B) Diffuse strong positive reaction to immunohistochemical stain for p53 in HGSC is shown (p53 immunohistochemical stain, ×200).

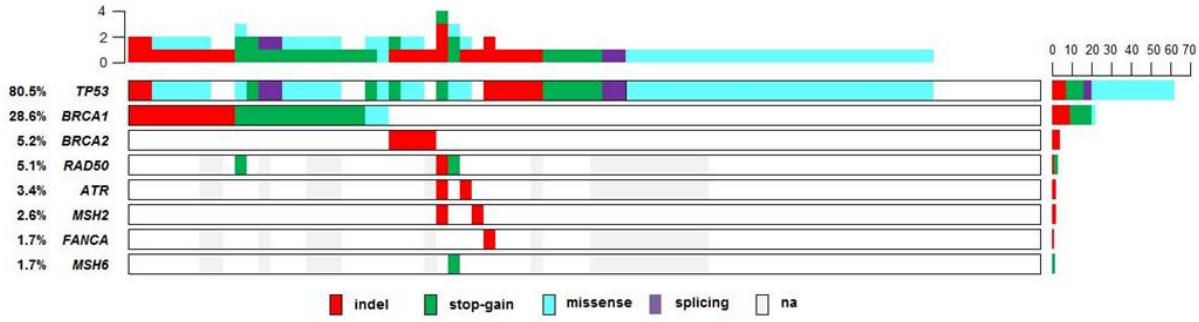


Fig. 2. Pathogenic variants (n=98) in 77 ovarian cancer patients. *TP53* was mutated in 80.5% of cases, the highest frequency among the 39 genes analyzed. *BRCA1* and *BRCA2* were mutated in 28.6% and 5.2% of cases, respectively. The color of each cell represents the type of variant: red, indel; green, stop-gain; sky blue, missense; purple, splicing; light gray, na (data not available); and white, wild type.

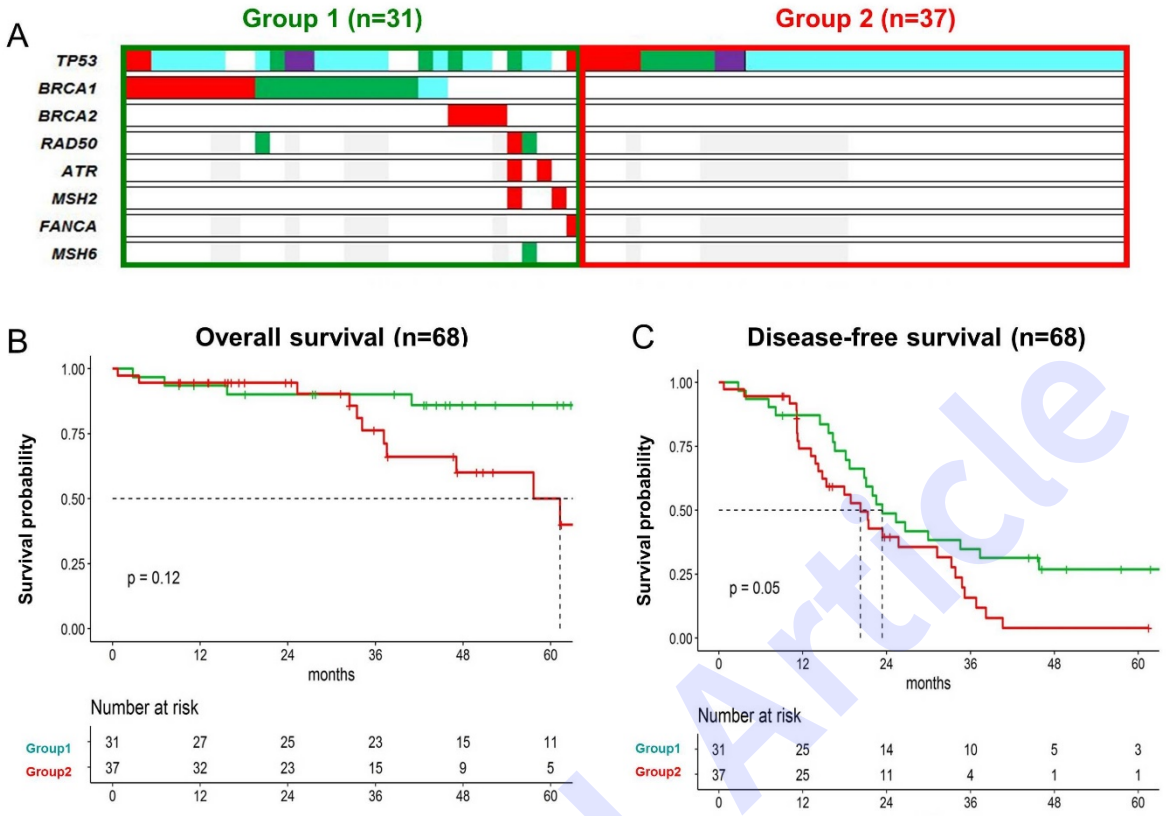


Fig. 3. The prognosis of three groups of patients, classified according to pathogenic variants. (A) Two groups were defined by their status for the pathogenic variants described in Fig. 1. Group 1 (green) consisted of patients with any pathogenic mutations in the 38 DNA damage repair (DDR) genes regardless of *TP53* mutation status. Group 2 (red) consists of patients with only *TP53* mutations. Overall survival and disease-free survival of the two groups are shown in B and C, respectively.

Table 1. Thirty-nine genes associated with hereditary ovarian cancer

Pathway	Gene
Hereditary breast and ovarian cancer syndrome	*BRCA1*, *BRCA2*
Homologous recombination pathway	*ATM*, *ATR*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAAP24*, *FAM175A* (*ABRAXAS1*), *FANCA*, *FANCB*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *SLX4* (*FANCP*), *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD52*, *RAD54B*, *RAD54L*
Mismatch repair	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *MLH3*
p53	*TP53*

Table 2. Samples and sequencing methods

Sample	**Sequencing method**	**No. of cases**
Fresh tumor tissue	Whole exome sequencing	59
FFPE tumor tissue	Panel sequencing	18
FFPE matched normal lymph node tissue for fresh tumor tissue	whole exome sequencing	48
Blood	Sanger sequencing	47

FFPE, formalin-fixed paraffin-embedded.

Table 3. Patient clinical characteristics

Characteristic	No. (%) (n=77)
Ethnicity	
Korean	73 (94.8)
Korean-Chinese	4 (5.2)
Age (years), median (range)	56 (36–82)
Cancer site	
Ovary	71 (92.2)
Fallopian tube	3 (3.9)
Peritoneum	3 (3.9)
Histology	
High-grade serous	73 (94.8)
Seromucinous	2 (2.6)
Carcinosarcoma	2 (2.6)
Stage	
I	2 (2.6)
II	6 (7.8)
III	59 (76.6)
IV	10 (13.0)
Breast cancer history	
Yes	6 (7.8)
No	71 (92.2)
Family history of BRCA-related cancer[a)]	
Yes	17 (22.1)
No	60 (77.9)
Residual mass (cm)	
< 1	67 (87.0)
≥ 1	10 (13.0)
Platinum sensitivity	
Sensitive	40 (64.5)
Resistant	16 (25.8)
Lost to follow-up	6 (9.7)
Status	
NED	15 (19.5)
AWD	35 (45.5)
Death	21 (27.3)
Lost to follow-up	6 (7.8)

NED, no evidence of disease; AWD, alive with disease. [a)]Family history of breast, peritoneal, ovarian, fallopian tube, pancreas, or prostate cancer in second degree relatives.

Table 4. Detected *BRCA1/2* mutations in 47 patients by germline and somatic genetic test

Case	Age (yr)	Cancer	Stage	Detected in blood and/or matched normal tissue		Detected in tumor		Germline/Somatic	Family history[a)]
				Gene	Mutation	Gene	Mutation		
OCA07	53	Ovary	III	*BRCA1*	c.2048delA	*BRCA1*	c.2048delA	Germline	+
Pat55	59	Ovary	III	*BRCA1*	c.2359delG	*BRCA1*	c.2359delG	Germline	–
OCA02	47	Ovary	III	*BRCA1*	c.928C>T	*BRCA1*	c.928C>T	Germline	–
Pat10	53	Ovary	III	*BRCA1*	c.1716dupA	*BRCA1*	c.1716dupA	Germline	+
Pat48	64	Ovary	III	*BRCA1*	c.3442G>T	*BRCA1*	c.3442G>T	Germline	+
Pat20	52	Ovary	III	*BRCA1*	c.3700_3704delGTAAA	*BRCA1*	c.3700_3704delGTAAA	Germline	+
Pat45	61	Ovary	III	*BRCA1*	c.922_924delAGCinsT	*BRCA1*	c.922_924delAGCinsT	Germline	+
Pat21	55	Ovary	III	*BRCA1*	c.4801A>T	*BRCA1*	c.4801A>T	Germline	+
Pat46	47	Ovary	III	*BRCA1*	c.390C>A	*BRCA1*	c.390C>A	Germline	+
Pat11	65	Ovary	III	*BRCA1*	c.390C>A	*BRCA1*	c.390C>A	Germline	+
Pat05	77	Ovary	IV	*BRCA2*	c.3860delA	*BRCA2*	c.3860delA	Germline	+
Pat08	55	Ovary	III	*BRCA2*	c.5576_5579delTTAA	*BRCA2*	c.5576_5579delTTAA	Germline	+
OCA18	57	FTC	III	*BRCA1*	c.3895C>T	*BRCA1*	c.3895C>T	Germline	+
OCA13	43	PPC	III	*BRCA1*	c.3813dupT	*BRCA1*	c.3813dupT	Germline	–
Pat41	58	Ovary	III	*BRCA1*	c.5496_5506delinsA	*BRCA1*	c.5496_5506delinsA	Germline	+

Pat49	48	Ovary	III	*BRCA1*	c.390C>A	*BRCA1*	c.390C>A	Germline	–
Pat47	49	Ovary	III	Not detected		*BRCA1*	c.4287C>A	Somatic	–
OCA16	55	Ovary	IV	Not detected		*BRCA1*	c.969_970delAA	Somatic	–
OCA19	52	FTC	III	Not detected		*BRCA1*	c.1813delG	Somatic	+
OCA14	50	PPC	III	Not detected		*BRCA2*	c.547delA	Somatic	+

FTC, fallopian tubal cancer; PPC, primary peritoneal cancer. [a)] Family history of BRCA-related cancer within second degree relatives and/or patient history of breast cancer; WES, whole exome sequencing

Table 5. Frequency of *BRCA* germline and somatic mutations in ovarian cancer reported in the literature

Cancer site	Histology	Nation of publication	Germline mutation	Somatic mutation	S/(S+G) ratio	G and S mutation	Study
Ovary	Serous and non-serous	USA	17/28 (60.7)	11/28 (39.3)	11/28 (39.3)	28/28 (100)	Hennessy (2010)
Ovary	Serous and non-serous	China	12/50 (24.0)	2/50 (4.0)	2/14 (14.3)	14/50 (28.0)	Zhao (2017)
Ovary	HGS	USA	51/316 (16.1)	21/316 (6.6)	21/71 (29.6)	71/316 (22.5)	TCGA (2011)
POFT	HGS	Canada	26/103 (25.2)	5/103 (4.9)	5/31 (16.1)	31/103 (30.1)	McAlpine (2012)
Ovary	HGS	Italy	10/47 (21.3)	3/47 (6.4)	3/13 (23.1)	13/47 (27.7)	Mafficini (2016)
Ovary	HGS	Taiwan	8/46 (17.4)	4/46 (8.7)	4/12 (33.3)	12/46 (26.1)	Chao (2016)
POFT	HGS	Korea	16/47 (34.0)	4/47 (8.5)	4/20 (20.0)	20/47 (42.6)	Present study (2019)
Total[a)]			111/559 (19.9)	37/559 (6.6)	37/147 (25.2)	147/559 (26.3)	

S, somatic; G, germline; HGS, high-grade serous; POFT, peritoneal/ovarian/fallopian tube cancer. [a)] Calculated from the five studies of HGS.

S1 Table. Ninety-eight pathogenic and likely pathogenic variants of 39 genes in 77 patients

Pat ID	Gene	Chromosome	Position	HGVSc	HGVSp	Mutation type	mRNA	Ref	Alt	Gene ID	QUAL	Depth	Allele freq	Pathogenicity	Somatic/ Germline	Methods for somatic calls
Pat12	ATR	chr3	142274783	c.2277delA	p.Ala760Leufs*11	Frameshift deletion	NM_001184.3	T	-	545	157	90	0.16	Novel	Somatic	Matched normal
Pat53	ATR	chr3	142217557	c.5440delA	p.Arg1814Glufs*10	Frameshift deletion	NM_001184.3	T	-	545	233	62	0.29	Novel	Somatic	Matched normal
Pat11	BRCA1	chr17	41256190	c.390C>A	p.Tyr130*	Stopgain	NM_007294.3	G	T	672	1395	60	0.95	Pathogenic	Germline	Matched normal
Pat46	BRCA1	chr17	41256190	c.390C>A	p.Tyr130*	Stopgain	NM_007294.3	G	T	672	2134	105	0.83	Pathogenic	Germline	Matched normal & BRCA sanger
Pat49	BRCA1	chr17	41256190	c.390C>A	p.Tyr130*	Stopgain	NM_007294.3	G	T	672	787	39	0.77	Pathogenic	Germline	Matched normal
Pat45	BRCA1	chr17	41246624	c.923_924delGC	p.Ser308Lysfs*11	Frameshift deletion	NM_007294.3	GC	-	672	3067	89	0.86	Pathogenic	Germline	Matched normal & BRCA sanger
OCA02	BRCA1	chr17	41246620	c.928C>T	p.Gln310*	Stopgain	NM_007294.3	G	A	672	4000	2533	0.65	Pathogenic	Germline	BRCA sanger
OCA16	BRCA1	chr17	41246578	c.969_970delAA	p.Ser324*	Stopgain	NM_007294.3	TT	-	672	4000	4217	0.67	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Somatic	BRCA sanger
Pat42	BRCA1	chr17	41246436	c.1112delC	p.Pro371Leufs*3	Frameshift deletion	NM_007294.3	G	-	672	1754	107	0.55	Pathogenic	Somatic	Matched normal & BRCA sanger
Pat10	BRCA1	chr17	41245832	c.1716dupA	p.Ser573Ilefs*13	Frameshift insertion	NM_007294.3	-	T	672	437	21	0.75	Pathogenic	Germline	Matched normal

OCA19	BRCA1	chr17	41245735	c.1813delG	p.Ala605Hisfs*7	Frameshift deletion	NM_007294.3	C	-	672	4000	2456	0.84	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Somatic	BRCA sanger
OCA07	BRCA1	chr17	41245500	c.2048delA	p.Lys683Serfs*18	Frameshift deletion	NM_007294.3	T	-	672	4000	2930	0.86	Pathogenic	Germline	BRCA sanger
Pat55	BRCA1	chr17	41245189	c.2359delG	p.Glu787Lysfs*5	Frameshift deletion	NM_007294.3	C	-	672	4669	169	0.85	Pathogenic	Germline	Matched normal
Pat60	BRCA1	chr17	41244528	c.3020delC	p.Ser1007*	Frameshift deletion	NM_007294.3	G	-	672	1459	41	0.95	Pathogenic	Germline	Matched normal
Pat48	BRCA1	chr17	41244106	c.3442G>T	p.Glu1148*	Stopgain	NM_007294.3	C	A	672	1269	57	0.88	Pathogenic	Germline	Matched normal & BRCA sanger
Pat20	BRCA1	chr17	41243844	c.3700_3704delTAAA	p.Val1234Glnfs*8	Frameshift deletion	NM_007294.3	TTTAC	-	672	4034	137	0.78	Pathogenic	Germline	Matched normal & BRCA sanger
OCA13	BRCA1	chr17	41243735	c.3813dupT	p.Asn1272*	Stopgain	NM_007294.3	-	A	672	4000	6841	0.79	Pathogenic	Germline	BRCA sanger
OCA18	BRCA1	chr17	41243653	c.3895C>T	p.Gln1299*	Stopgain	NM_007294.3	G	A	672	4000	2566	0.89	Pathogenic	Germline	BRCA sanger
Pat36	BRCA1	chr17	41243557	c.3991C>T	p.Glu1331*	Stopgain	NM_007294.3	G	A	672	4649	210	0.90	Pathogenic	Do not know	
Pat47	BRCA1	chr17	41234491	c.4287C>A	p.Tyr1429*	Stopgain	NM_007294.3	G	T	672	832	40	0.75	Pathogenic	Somatic	Matched normal
Pat21	BRCA1	chr17	41223130	c.4801A>T	p.Lys1601*	Stopgain	NM_007294.3	T	A	672	3486	132	0.96	Pathogenic	Germline	Matched normal & BRCA sanger
Pat23	BRCA1	chr17	41201205	c.5339T>C	p.Leu1780Pro	missense	NM_007294.3	A	G	672	2068	102	0.81	Likely pathogenic	Germline	Matched normal
Pat28	BRCA1	chr17	41201205	c.5339T>C	p.Leu1780Pro	missense	NM_007294.3	A	G	672	2123	129	0.70	Likely pathogenic	Germline	Matched normal

Pat41	BRCA1	chr17	41197781	c.5496_5506delGGTGACCCGAGinsA	p.Val1833Serfs*7	in-frame deletion	NM_007294.3	CTCGGGTCACC	T	672	1386	47	0.74	Pathogenic	Germline	Matched normal
OCA14	BRCA2	chr13	32900664	c.547delA	p.Ser183Valfs*2	Frameshift deletion	NM_000059.3	A	-	675	4000	6675	0.59	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Somatic	BRCA sanger
Pat54	BRCA2	chr13	32911057	c.2567delA	p.Asn856Ilefs*2	Frameshift deletion	NM_000059.3	A	-	675	934	47	0.69	Novel	Germline	Matched normal
Pat05	BRCA2	chr13	32912346	c.3860delA	p.Asn1287Ilefs*6	Frameshift deletion	NM_000059.3	A	-	675	433	27	0.82	Pathogenic	Germline	BRCA sanger
Pat08	BRCA2	chr13	32914066	c.5576_5579delTTAA	p.Ile1859Lysfs*3	Frameshift deletion	NM_000059.3	AATT	-	675	783	23	0.87	Pathogenic	Germline	Matched normal & BRCA sanger
Pat17	FANCA	chr16	89809249	c.3720_3724delAAACA	p.Glu1240Aspfs*36	Frameshift deletion	NM_000135.2	TGTTT	-	2175	680	92	0.25	pathogenic	Germline	Matched normal
Pat53	MSH2	chr2	47637247	c.387_388delTC	p.Gln130Valfs*2	Frameshift deletion	NM_000251.2	TC	-	4436	2101	71	0.83	Pathogenic	Germline	Matched normal
Pat07	MSH2	chr2	47708005	c.2633_2634delAG	p.Glu878Alafs*3	Frameshift deletion	NM_000251.2	AG	-	4436	1323	83	0.49	Pathogenic	Germline	Matched normal
Pat14	MSH6	chr2	48033753	c.3964G>T	p.Glu1322*	Stopgain	NM_000179.2	G	T	2956	86	105	0.15	Pathogenic	Somatic	Matched normal
Pat21	RAD50	chr5	131925407	c.1330G>T	p.Glu444*	Stopgain	NM_005732.3	G	T	10111	189	26	0.35	Novel	Somatic	Matched normal
Pat53	RAD50	chr5	131931451	c.2165delA	p.Lys722Argfs*14	Frameshift deletion	NM_005732.3	A	-	10111	1886	146	0.70	Pathogenic	Somatic	Matched normal
Pat14	RAD50	chr5	131953844	c.3247G>T	p.Glu1083*	Stopgain	NM_005732.3	G	T	10111	71	45	0.18	Novel	Somatic	Matched normal
OCA01	TP53	chr17	7579722	c.97-1G>T	-	Splicing	NM_000546.5	C	T	7157	4000	2969	0.93	Pathogenic	Do not know	
Pat41	TP53	chr17	7579573	c.114delA	p.Ala39Glnfs*5	Frameshift deletion	NM_000546.5	T	-	7157	1111	38	0.92	Novel	Somatic	Matched normal

Pat17	TP53	chr17	7579513	c.153_174delACAATGGTTCACTGAAGACCCA	p.Gln52Valfs*64	Frameshift deletion	NM_000546.5	TGGGTCTTCAGTGAACCATTGT	-	7157	416	30	0.41	Novel	Somatic	Matched normal
Pat36	TP53	chr17	7579414	c.273G>A	p.Trp91*	Stopgain	NM_000546.5	C	T	7157	392	22	0.77	Pathogenic	Do not know	
OCA15	TP53	chr17	7578539	c.391A>T	p.Asn131Tyr	Missense	NM_000546.5	T	A	7157	4000	4377	0.69	Pathogenic	Do not know	
OCA13	TP53	chr17	7578535	c.395A>G	p.Lys132Arg	Missense	NM_000546.5	T	C	7157	4000	4395	0.43	Likely pathogenic	Do not know	
Pat44	TP53	chr17	7578535	c.395A>G	p.Lys132Arg	Missense	NM_000546.5	T	C	7157	347	51	0.31	Likely pathogenic	Somatic	Matched normal
Pat11	TP53	chr17	7578535	c.395A>G	p.Lys132Arg	Missense	NM_000546.5	T	C	7157	1718	82	0.76	Likely pathogenic	Somatic	Matched normal
OCA07	TP53	chr17	7578525	c.405C>G	p.Cys135Trp	Missense	NM_000546.5	G	C	7157	4000	8223	0.78	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
OCA05	TP53	chr17	7578524	c.406C>T	p.Gln136*	Stopgain	NM_000546.5	G	A	7157	4000	3612	0.80	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
Pat22	TP53	chr17	7578469	c.450_460delACCCCCGCCCG	p.Pro151Hisfs*26	Frameshift deletion	NM_000546.5	CGGGCGGGGGT	-	7157	1124	49	0.60	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Somatic	Matched normal
OCA12	TP53	chr17	7578478	c.452C>G	p.Pro151Arg	Missense	NM_000546.5	G	C	7157	4000	4106	0.76	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
Pat24	TP53	chr17	7578442	c.488A>G	p.Tyr163Cys	Missense	NM_000546.5	T	C	7157	798	54	0.59	Pathogenic	Somatic	Matched normal

Pat61	TP53	chr17	7578407	c.523C>G	p.Arg175Gly	Missense	NM_000546.5	G	C	7157	1743	76	0.83	Pathogenic	Somatic	Matched normal
OCA09	TP53	chr17	7578235	c.497A>G	p.Tyr166Cys	Missense	NM_001126118.1	T	C	7157	4000	3405	0.58	Likely pathogenic	Do not know	
Pat10	TP53	chr17	7578406	c.524G>A	p.Arg175His	Missense	NM_000546.5	C	T	7157	1076	48	0.81	Pathogenic	Somatic	Matched normal
Pat18	TP53	chr17	7578406	c.524G>A	p.Arg175His	Missense	NM_000546.5	C	T	7157	867	40	0.78	Pathogenic	Somatic	Matched normal
Pat25	TP53	chr17	7578403	c.527G>T	p.Cys176Phe	Missense	NM_000546.5	C	A	7157	722	38	0.74	Likely pathogenic	Do not know	
OCA06	TP53	chr17	7578394	c.536A>G	p.His179Arg	Missense	NM_000546.5	T	C	7157	4000	3302	0.70	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
Pat52	TP53	chr17	7578392	c.538G>A	p.Glu180Lys	Missense	NM_000546.5	C	T	7157	212	37	0.30	Likely pathogenic	Do not know	
OCA18	TP53	chr17	7578283	c.566C>T	p.Ala189Val	Missense	NM_000546.5	G	A	7157	4000	3393	0.93	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
Pat58	TP53	chr17	7578271	c.578A>G	p.His193Arg	Missense	NM_000546.5	T	C	7157	608	64	0.44	Likely pathogenic	Do not know	
Pat55	TP53	chr17	7578271	c.578A>G	p.His193Arg	Missense	NM_000546.5	T	C	7157	1319	77	0.70	Likely pathogenic	Somatic	Matched normal
Pat21	TP53	chr17	7578269	c.580C>T	p.Leu194Phe	Missense	NM_000546.5	G	A	7157	1507	71	0.85	Likely pathogenic	Somatic	Matched normal
OCA03	TP53	chr17	7578265	c.584T>C	p.Ile195Thr	Missense	NM_000546.5	A	G	7157	4000	5700	0.52	Pathogenic	Do not know	
Pat20	TP53	chr17	7578265	c.584T>C	p.Ile195Thr	Missense	NM_000546.5	A	G	7157	886	71	0.52	Pathogenic	Somatic	Matched normal
Pat45	TP53	chr17	7578265	c.584T>C	p.Ile195Thr	Missense	NM_000546.5	A	G	7157	1088	71	0.61	Pathogenic	Somatic	Matched normal
Pat57	TP53	chr17	7578262	c.587G>C	p.Arg196Pro	Missense	NM_000546.5	C	G	7157	1140	108	0.46	Likely pathogenic	Somatic	Matched normal

Pat42	TP53	chr17	7578249	c.599delA	p.Asn200Ilefs*47	Frameshift deletion	NM_000546.5	T	-	7157	1150	75	0.56	pathogenic	Somatic	Matched normal
Pat16	TP53	chr17	7578219	c.629delA	p.Asn210Thrfs*37	Frameshift deletion	NM_000546.5	T	-	7157	1107	93	0.43	Novel	Somatic	Matched normal
Pat53	TP53	chr17	7578212	c.637C>T	p.Arg213*	Stopgain	NM_000546.5	G	A	7157	1711	81	0.83	Pathogenic	Somatic	Matched normal
Pat12	TP53	chr17	7578190	c.659A>G	p.Tyr220Cys	Missense	NM_000546.5	T	C	7157	109	56	0.18	Pathogenic	Somatic	Matched normal
Pat15	TP53	chr17	7578190	c.659A>G	p.Tyr220Cys	Missense	NM_000546.5	T	C	7157	899	47	0.72	Pathogenic	Somatic	Matched normal
Pat35	TP53	chr17	7578190	c.659A>G	p.Tyr220Cys	Missense	NM_000546.5	T	C	7157	432	69	0.28	Pathogenic	Do not know	
OCA02	TP53	chr17	7577610	c.673-2A>T	-	splicing	NM_000546.5	T	C	7157	4000	6469	0.49	Pathogenic	Do not know	
OCA10	TP53	chr17	7577609	c.673-1G>A	-	splicing	NM_000546.5	C	T	7157	4000	5122	0.48	Pathogenic	Do not know	
Pat43	TP53	chr17	7577607	c.673dupG	p.Val225Glyfs*4	Frameshift insertion	NM_000546.5	-	C	7157	1587	74	0.73	Novel	Somatic	Matched normal
OCA08	TP53	chr17	7577593	c.688_689insTGTA	p.Thr230Metfs*11	Frameshift insertion	NM_000546.5	-	TACA	7157	4000	3255	0.95	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
Pat29	TP53	chr17	7577581	c.700T>C	p.Tyr234His	Missense	NM_000546.5	A	G	7157	1915	115	0.70	Pathogenic	Somatic	Matched normal
Pat54	TP53	chr17	7577580	c.701A>G	p.Tyr234Cys	Missense	NM_000546.5	T	C	7157	872	61	0.62	Pathogenic	Somatic	Matched normal
Pat31	TP53	chr17	7577570	c.711G>C	p.Met237Ile	Missense	NM_000546.5	C	G	7157	976	41	0.80	pathogenic	Do not know	
Pat49	TP53	chr17	7577570	c.711G>A	p.Met237Ile	Missense	NM_000546.5	C	T	7157	856	44	0.84	Likely pathogenic	Somatic	Matched normal
Pat09	TP53	chr17	7577547	c.734G>A	p.Gly245Asp	Missense	NM_000546.5	C	T	7157	1038	85	0.54	Pathogenic	Somatic	Matched normal
Pat27	TP53	chr17	7577539	c.742C>T	p.Arg248Trp	Missense	NM_000546.5	G	A	7157	470	40	0.51	Pathogenic	Somatic	Matched normal
OCA18	TP53	chr17	7577538	c.743G>A	p.Arg248Gln	Missense	NM_000546.5	C	T	7157	4000	13331	0.89	Pathogenic	Do not know	

Pat28	TP53	chr17	7577536	c.745A>G	p.Arg249Gly	Missense	NM_000546.5	T	C	7157	649	60	0.47	Somatic: pathogenic	Somatic	Matched normal
Pat33	TP53	chr17	7577511	c.770T>G	p.Leu257Arg	Missense	NM_000546.5	A	C	7157	594	33	0.72	Likely pathogenic	Somatic	Matched normal
Pat02	TP53	chr17	7577507	c.774A>C	p.Glu258Asp	Missense	NM_000546.5	T	G	7157	424	28	0.67	Pathogenic	Somatic	Matched normal
OCA11	TP53	chr17	7577153	c.785G>T	p.Gly262Val	Missense	NM_000546.5	C	A	7157	4000	3643	0.57	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
OCA16	TP53	chr17	7577153	c.785G>T	p.Gly262Val	Missense	NM_000546.5	C	A	7157	4000	3194	0.58	Pathogenic (Loss-of-Function in Oncomine Knowledgebase)	Do not know	
Pat05	TP53	chr17	7577124	c.814G>C	p.Val272Leu	Missense	NM_000546.5	C	G	7157	679	21	1.00	Pathogenic	Somatic	Matched normal
Pat38	TP53	chr17	7577120	c.818G>A	p.Arg273His	Missense	NM_000546.5	C	T	7157	60	24	0.75	Pathogenic	Do not know	
Pat14	TP53	chr17	7577117	c.821T>G	p.Val274Gly	Missense	NM_000546.5	A	C	7157	36	29	0.83	Pathogenic	Somatic	Matched normal
Pat39	TP53	chr17	7577108	c.830G>T	p.Cys277Phe	Missense	NM_000546.5	C	A	7157	459	36	0.50	Pathogenic	Somatic	Matched normal
Pat13	TP53	chr17	7577106	c.832C>A	p.Pro278Thr	Missense	NM_000546.5	G	T	7157	1031	44	0.89	Pathogenic	Somatic	Matched normal
Pat06	TP53	chr17	7577046	c.892G>T	p.Glu298*	Stopgain	NM_000546.5	C	A	7157	3071	117	0.93	Pathogenic	Somatic	Matched normal
Pat23	TP53	chr17	7577022	c.916C>T	p.Arg306*	Stopgain	NM_000546.5	G	A	7157	3269	161	0.86	Pathogenic	Somatic	Matched normal
Pat40	TP53	chr17	7577022	c.916C>T	p.Arg306*	Stopgain	NM_000546.5	G	A	7157	923	68	0.60	Pathogenic	Somatic	Matched normal
Pat08	TP53	chr17	7577022	c.916C>T	p.Arg306*	Stopgain	NM_000546.5	G	A	7157	1235	63	0.78	Pathogenic	Somatic	Matched normal
Pat47	TP53	chr17	7576928	c.920-2A>G		splicing	NM_000546.5	T	C	7157	1030	45	0.80	Likely pathogenic	Somatic	Matched normal
OCA04	TP53	chr17	7574018	c.1009C>T	p.Arg337Cys	Missense	NM_000546.5	G	A	7157	4000	3729	0.63	Pathogenic	Do not know	

Pat26	TP53	chr17	7574000	c.1027G>T	p.Glu343*	Stopgain	NM_000546.5	C	A	7157	224	39	0.31	Novel	Somatic	Matched normal
Pat30	TP53	chr17	7573991	c.1035dupT	p.Glu346*	Stopgain	NM_000546.5	-	A	7157	593	64	0.35	Novel	Somatic	Matched normal

S2 Table. Sixty-three variants unknown significance in 77 patients

Pat ID	Gene	Chromosome	Position	HGVSc	HGVSp	Mutation type	mRNA	Ref	Alt	Gene ID	QUAL	Depth	Allele freq	Pathogenicity	Somatic/ Germline	Methods for Somatic calls
OCA12	ATM	chr11	108115553	c.701C>G	p.Ala234Gly	Missense	NM_000051.3	C	G	472	4000	3926	0.344	VUS	do not know	
Pat32	ATM	chr11	108122697	c.1741T>G	p.Leu581Val	Missense	NM_000051.3	T	G	472	554	45	0.47	VUS	do not know	
Pat53	ATM	chr11	108151786	c.3467C>T	p.Thr1156Met	Missense	NM_000051.3	C	T	472	347	94	0.2	VUS	Somatic	Matched normal
Pat60	ATM	chr11	108155170	c.3963G>A	p.Met1321Ile	Missense	NM_000051.3	G	A	472	1891	173	0.49	VUS	Germline	Matched normal
Pat27	ATM	chr11	108173629	c.5369A>G	p.Asp1790Glu	Missense	NM_000051.3	A	G	472	150	56	0.2	VUS	Germline	Matched normal
Pat50	ATM	chr11	108206666	c.8246A>T	p.Lys2749Ile	Missense	NM_000051.3	A	T	472	616	58	0.48	VUS	do not know	
Pat13	ATR	chr3	142285088	c.167T>C	p.Val56Ala	Missense	NM_001184.3	A	G	545	3243	101	0.97	VUS	Germline	Matched normal
Pat43	BARD1	chr2	215645670	c.928T>C	p.Ser310Pro	Missense	NM_000465.3	A	G	580	1633	111	0.52	VUS	Germline	Matched normal
Pat47	BARD1	chr2	215632249	c.1525A>G	p.Ile509Val	Missense	NM_000465.3	T	C	580	2709	152	0.48	VUS	Germline	Matched normal
Pat18	BRCA1	chr17	41258531	c.154C>T	p.Leu52Phe	Missense	NM_007294.3	G	A	672	301	22	0.59	VUS	Germline	Matched normal
Pat45	BRCA1	chr17	41246626	c.922A>T	p.Ser308Cys	Missense	NM_007294.3	T	A	672	3076	86	0.87	VUS	Germline	Matched normal
Pat21	BRCA1	chr17	41244377	c.3171T>G	p.Ser1057Arg	Missense	NM_007294.3	A	C	672	4753	173	0.92	VUS	Somatic	Matched normal
Pat13	BRCA2	chr13	32899267	c.371T>C	p.Met124Thr	Missense	NM_000059.3	T	C	675	390	29	0.59	VUS	Germline	Matched normal
Pat60	BRCA2	chr13	32900742	c.623T>G	p.Val208Gly	Missense	NM_000059.3	T	G	675	2912	110	0.95	VUS	Germline	Matched normal
Pat27	BRCA2	chr13	32914643	c.6151A>G	p.Asn2051Asp	Missense	NM_000059.3	A	G	675	911	33	0.85	VUS	Germline	Matched normal
Pat34	BRCA2	chr13	32914814	c.6322C>T	p.Arg2108Cys	Missense	NM_000059.3	C	T	675	1459	50	0.98	VUS	Germline	Matched normal

Pat41	BRCA2	chr13	32930651	c.7522G>A	p.Gly2508Ser	Missense	NM_000059.3	G	A	675	1895	81	0.89	VUS	Germline	Matched normal
Pat36	BRCA2	chr13	32931894	c.7633G>A	p.Val2545Ile	Missense	NM_000059.3	G	A	675	340	25	0.48	VUS	do not know	
OCA04	BRCA2	chr13	32945231	c.8626C>A	p.His2876Asn	Missense	NM_000059.3	C	A	675	1138	765	0.312	VUS	do not know	
Pat09	BRCA2	chr13	32972800	c.10150C>T	p.Arg3384*	Stopgain	NM_000059.3	C	T	675	675	75	0.4	VUS	Germline	Matched normal & BRCA sanger
Pat55	BRIP1	chr17	59924476	c.613T>C	p.Phe205Leu	Missense	NM_032043.2	A	G	83990	1303	51	0.88	VUS	Germline	Matched normal
Pat08	BRIP1	chr17	59793364	c.2440C>T	p.Arg814Cys	Missense	NM_032043.2	G	A	83990	2603	111	0.9	VUS	Germline	Matched normal
Pat07	BRIP1	chr17	59760967	c.3440delA	p.Asn1147Metfs*3	Frameshift deletion	NM_032043.2	T	-	83990	224	53	0.29	VUS	Somatic	Matched normal
Pat60	CHEK2	chr22	29091133	c.1486G>C	p.Ala496Pro	Missense	NM_007194.3	C	G	11200	1177	40	0.98	VUS	Germline	Matched normal
Pat47	FANCA	chr16	89877351	c.412A>G	p.Thr138Ala	Missense	NM_000135.2	T	C	2175	1185	104	0.47	VUS	Germline	Matched normal
Pat19	FANCA	chr16	89838136	c.2101A>G	p.Lys701Glu	Missense	NM_000135.2	T	C	2175	372	46	0.39	VUS	Do not know	
Pat07	FANCB	chrX	14863068	c.1837C>T	p.Arg613Cys	Missense	NM_152633.3	G	A	2187	140	71	0.2	VUS	Somatic	Matched normal
Pat09	FANCB	chrX	14862803	c.1987A>G	p.Thr663Ala	Missense	NM_152633.3	T	C	2187	1320	113	0.46	VUS	Germline	Matched normal
Pat59	FANCC	chr9	97879669	c.1000C>T	p.Arg334Trp	Missense	NM_000136.2	G	A	2176	1481	109	0.59	VUS	Somatic	Matched normal
Pat19	FANCD2	chr3	10108987	c.2480A>C	p.Glu827Ala	Missense	NM_033084.4	A	C	2177	1523	158	0.41	VUS	do not know	
Pat48	FANCE	chr6	35427198	c.1204C>T	p.Leu402Phe	Missense	NM_021922.2	C	T	2178	2670	151	0.66	VUS	Germline	Matched normal
Pat58	FANCG	chr9	35079514	c.8G>T	p.Arg3Leu	Missense	NM_004629.1	C	A	2189	1534	129	0.51	VUS	do not know	
Pat21	FANCG	chr9	35077395	c.512G>A	p.Ser171Asn	Missense	NM_004629.1	C	T	2189	1987	164	0.5	VUS	Somatic	Matched normal

Pat29	FANCG	chr9	35076561	c.944G>A	p.Ser315Asn	Missense	NM_004629.1	C	T	2189	1122	105	0.45	VUS	Germline	Matched normal
Pat29	FANCI	chr15	89804853	c.326C>G	p.Ala109Gly	Missense	NM_001113378.1	C	G	55215	1339	123	0.41	VUS	Germline	Matched normal
Pat59	FANCI	chr15	89836203	c.2200A>G	p.Ser734Gly	Missense	NM_001113378.1	A	G	55215	222	22	0.41	VUS	Somatic	Matched normal
Pat23	FANCI	chr15	89856189	c.3706G>A	p.Val1236Ile	Missense	NM_001113378.1	G	A	55215	5357	216	0.89	VUS	Germline	Matched normal
Pat54	FANCI	chr15	89858603	c.3907G>A	p.Glu1303Lys	Missense	NM_001113378.1	G	A	55215	2340	77	0.8	VUS	Germline	Matched normal
Pat43	FANCM	chr14	45642337	c.2240A>G	p.His747Arg	Missense	NM_020937.3	A	G	57697	2494	180	0.61	VUS	Germline	Matched normal
Pat56	FANCM	chr14	45642337	c.2240A>G	p.His747Arg	Missense	NM_020937.3	A	G	57697	536	106	0.26	VUS	Do not know	
Pat52	FANCM	chr14	45650900	c.4378A>C	p.Ile1460Leu	Missense	NM_020937.3	A	C	57697	307	20	0.6	VUS	Do not know	
Pat03	FANCM	chr14	45657062	c.4751C>A	p.Thr1584Lys	Missense	NM_020937.3	C	A	57697	570	39	0.62	VUS	Somatic	Matched normal
Pat32	MLH1	chr3	37035090	c.52C>G	p.Arg18Gly	Missense	NM_000249.3	C	G	4292	1397	133	0.47	VUS	Do not know	
Pat12	MLH3	chr14	75498844	c.3754A>G	p.Lys1252Glu	Missense	NM_001040108.1	T	C	27030	251	116	0.17	VUS	Somatic	Matched normal
Pat08	MSH6	chr2	48023174	c.599C>T	p.Ser200Leu	Missense	NM_000179.2	C	T	2956	743	85	0.44	VUS	Somatic	Matched normal
Pat27	MSH6	chr2	48026598	c.1476G>A	p.Met492Ile	Missense	NM_000179.2	G	A	2956	821	79	0.47	VUS	Germline	Matched normal
Pat55	MSH6	chr2	48028233	c.3111C>A	p.Phe1037Leu	Missense	NM_000179.2	C	A	2956	438	164	0.2	VUS	Somatic	Matched normal
Pat60	MSH6	chr2	48030631	c.3245C>T	p.Pro1082Leu	Missense	NM_000179.2	C	T	2956	2860	279	0.45	VUS	Germline	Matched normal
Pat58	MSH6	chr2	48033468	c.3772C>G	p.Gln1258Glu	Missense	NM_000179.2	C	G	2956	1075	71	0.52	VUS	Do not know	
Pat53	NBN	chr8	90990521	c.511A>G	p.Ile171Val	Missense	NM_002485.4	T	C	4683	392	62	0.31	VUS	Germline	Matched normal
Pat58	NBN	chr8	90965716	c.1601A>C	p.Asn534Thr	Missense	NM_002485.4	T	G	4683	107	70	0.13	VUS	Do not know	
Pat48	PALB2	chr16	23647149	c.718C>A	p.Pro240Thr	Missense	NM_024675.3	G	T	79728	2440	193	0.53	VUS	Germline	Matched normal

Pat48	PALB2	chr16	23632742	c.3054G>C	p.Glu1018Asp	Missense	NM_024675.3	C	G	79728	974	84	0.5	VUS	Germline	Matched normal
Pat03	PALB2	chr16	23614877	c.3464C>G	p.Ser1155Cys	Missense	NM_024675.3	G	C	79728	665	66	0.44	VUS	Somatic	Matched normal
Pat47	PMS2	chr7	6017389	c.2276-1G>C		splicing	NM_000535.6	C	G	5395	598	65	0.42	VUS	Somatic	Matched normal
Pat53	RAD50	chr5	131924538	c.1211A>G	p.Gln404Arg	Missense	NM_005732.3	A	G	10111	1137	129	0.4	VUS	Germline	Matched normal
Pat47	RAD50	chr5	131938993	c.2209C>G	p.Gln737Glu	Missense	NM_005732.3	C	G	10111	178	23	0.30	VUS	Germline	Matched normal
Pat13	RAD51D	chr17	33428027	c.932T>A	p.Ile311Asn	Missense	NM_002878.3	A	T	5892	1015	40	0.93	VUS	Germline	Matched normal
Pat47	RAD52	chr12	1040434	c.138G>C	p.Arg46Ser	Missense	NM_134424.3	C	G	5893	863	42	0.76	VUS	Germline	Matched normal
Pat44	RAD52	chr12	1040408	c.164G>A	p.Arg55His	Missense	NM_134424.3	C	T	5893	461	51	0.4	VUS	Germline	Matched normal
Pat36	RAD54B	chr8	95403925	c.1721G>A	p.Gly574Glu	Missense	NM_012415.3	C	T	25788	2279	272	0.39	VUS	Do not know	
Pat14	SLX4	chr16	3640200	c.3439G>A	p.Glu1147Lys	Missense	NM_032444.3	C	T	84464	69	73	0.12	VUS	Somatic	Matched normal
Pat54	SLX4	chr16	3632671	c.5177C>T	p.Ala1726Val	Missense	NM_032444.3	G	A	84464	2142	123	0.74	VUS	Germline	Matched normal

S3 Table. Summary of variants in each patient

Pat ID	BRCA1/2 pathogenic mutations	non-BRCA1/2 pathogenic mutations	Variants unknown significance	Detection method	Target Coverage	TP53 Coverage
OCA01	-	TP53 (c.97-1G>T)	-	Panel	2876.0	2613.9
OCA02	BRCA1 (c.928C>T, p.Gln310*)	TP53 (c.673-2A>T)	-	Panel	2961.0	6776.0
OCA03	-	TP53 (c.584T>C, p.Ile195Thr)	-	Panel	3144.0	3735.2
OCA04	-	TP53 (c.1009C>T, p.Arg337Cys)	BRCA2 (c.8626C>A, p.His2876Asn)	Panel	3877.0	3916.0
OCA05	-	TP53 (c.406C>T, p.Gln136*)	-	Panel	2906.0	3231.6
OCA06	-	TP53 (c.536A>G, p.His179Arg)	-	Panel	3005.0	4233.2
OCA07	BRCA1 (c.2048delA, p.Lys683Serfs*18)	TP53 (c.405C>G, p.Cys135Trp)	-	Panel	3499.0	6819.1
OCA08	-	TP53 (c.688_689insTGTA, p.Thr230Metfs*11)	-	Panel	3729.0	3574.0
OCA09	-	TP53 (c.497A>G, p.Tyr166Cys)	-	Panel	3156.0	2927.0
OCA10	-	TP53 (c.673-1G>A)	-	Panel	4544.0	4781.4
OCA11	-	TP53 (c.785G>T, p.Gly262Val)	-	Panel	3665.0	3915.4
OCA12	-	TP53 (c.452C>G, p.Pro151Arg)	ATM (c.701C>G, p.Ala234Gly)	Panel	4204.0	5910.5
OCA13	BRCA1 (c.3813dupT, p.Asn1272*)	TP53 (c.395A>G , p.Lys132Arg)	-	Panel	4083.0	4127.5
OCA14	BRCA2 (c.547delA, p.Ser183Valfs*2)	-	-	Panel	5790.0	6547.5
OCA15	-	TP53 (c.391A>T, p.Asn131Tyr)	-	Panel	2945.0	3398.2
OCA16	BRCA1 (c.969_970delAA, p.Ser324*)	TP53 (c.785G>T, p.Gly262Val)	-	Panel	3765.0	3564.9
OCA18	BRCA1 (c.3895C>T, p.Gln1299*)	TP53 (c.743G>A, p.Arg248Gln), TP53 (c.566C>T, p.Ala189Val)	-	Panel	3511.0	7567.9
OCA19	BRCA1 (c.1813delG, p.Ala605Hisfs*7)	-	-	Panel	2706.0	4152.1
Pat02	-	TP53 (c.774A>C, p.Glu258Asp)	-	WES	90.1	35.4

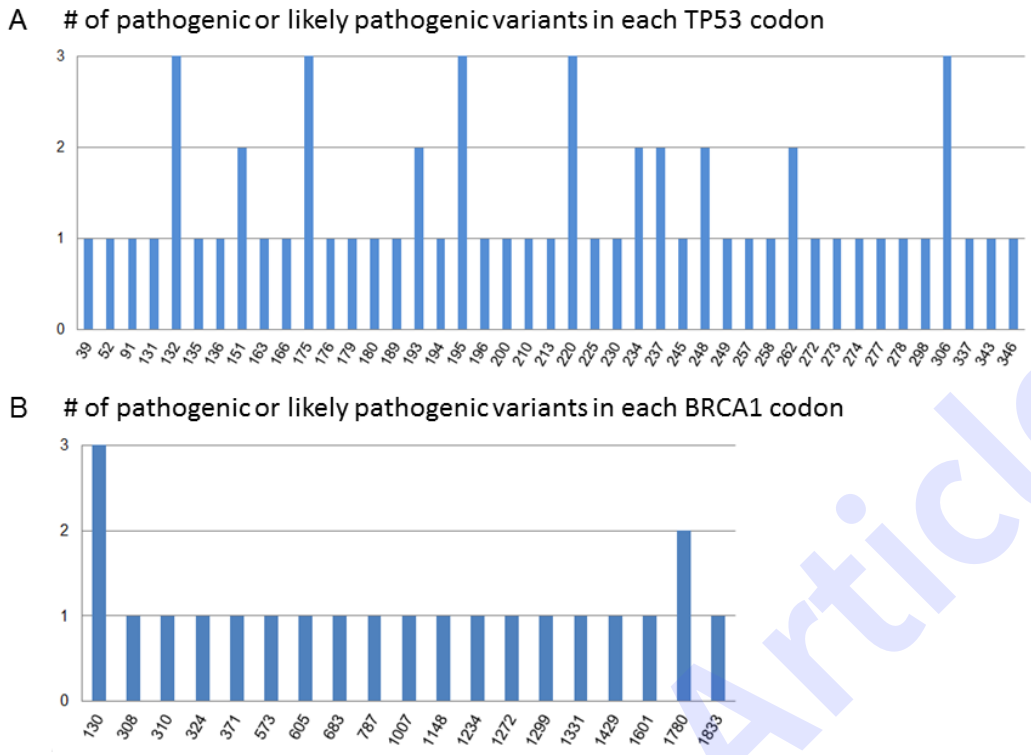
Pat03	-	-	FANCM (c.4751C>A, p.Thr1584Lys), PALB2 (c.3464C>G, p.Ser1155Cys)	WES	96.7	47.2
Pat05	BRCA2 (c.3860delA, p.Asn1287Ilefs*6)	TP53 (c.814G>C, p.Val272Leu)	-	WES	108.7	52.1
Pat06	-	TP53 (c.892G>T, p.Glu298*)	-	WES	102.9	73.8
Pat07	-	MSH2 (c.2633_2634delAG, p.Glu878Alafs*3)	FANCB (c.1837C>T, p.Arg613Cys), BRIP1 (c.3440delA, p.Asn1147Metfs*3)	WES	114.6	86.3
Pat08	BRCA2 (c.5576_5579delTTAA, p.Ile1859Lysfs*3)	TP53 (c.916C>T, p.Arg306*)	BRIP1 (c.2440C>T, p.Arg814Cys), MSH6 (c.599C>T, p.Ser200Leu)	WES	100.0	38.6
Pat09		TP53 (c.734G>A, p.Gly245Asp)	BRCA2 (c.10150C>T, p.Arg3384*), FANCB (c.1987A>G, p.Thr663Ala)	WES	92.0	84.6
Pat10	BRCA1 (c.1716dupA, p.Ser573Ilefs*13)	TP53 (c.524G>A, p.Arg175His)	-	WES	91.6	64.7
Pat11	BRCA1 (c.390C>A, p.Tyr130*)	TP53 (c.395A>G, p.Lys132Arg)	-	WES	96.2	91.8
Pat12	-	ATR (c.2277delA, p.Ala760Leufs*11), TP53 (c.659A>G, p.Tyr220Cys)	MLH3 (c.3754A>G, p.Lys1252Glu)	WES	89.0	54.2
Pat13	-	TP53 (c.832C>A, p.Pro278Thr)	ATR (c.167T>C, p.Val56Ala), BRCA2 (c.371T>C, p.Met124Thr), RAD51D (c.932T>A, p.Ile311Asn)	WES	93.2	75.3
Pat14	-	MSH6 (c.3964G>T, p.Glu1322*), RAD50 (c.3247G>T, p.Glu1083*), TP53 (c.821T>G, p.Val274Gly)	SLX4 (c.3439G>A, p.Glu1147Lys)	WES	96.4	67.8
Pat15	-	TP53 (c.659A>G, p.Tyr220Cys)	-	WES	92.6	43.2
Pat16	-	TP53 (c.629delA, p.Asn210Thrfs*37)	-	WES	83.8	65.0
Pat17	-	FANCA (c.3720_3724delAAACA, p.Glu1240Aspfs*36), TP53 (c.153_174delACAATGGTTCACTGAAGACCCA, p.Gln52Valfs*64)	-	WES	99.9	50.1

Pat18	-	TP53 (c.524G>A, p.Arg175His)	BRCA1 (c.154C>T, p.Leu52Phe)	WES	98.0	49.2
Pat19	-	-	FANCA (c.2101A>G, p.Lys701Glu), FANCD2 (c.2480A>C, p.Glu827Ala)	WES	97.8	50.2
Pat20	BRCA1 (c.3700_3704delTAAA, p.Val1234Glnfs*8)	TP53 (c.584T>C, p.Ile195Thr)		WES	97.2	64.2
Pat21	BRCA1 (c.4801A>T, p.Lys1601*)	RAD50 (c.1330G>T, p.Glu444*), TP53 (c.580C>T, p.Leu194Phe)	BRCA1 (c.3171T>G, p.Ser1057Arg), FANCG (c.512G>A, p.Ser171Asn)	WES	105.4	48.6
Pat22	-	TP53 (c.450_460delACCCCCGCCCG, p.Pro151Hisfs*26)	-	WES	102.3	47.0
Pat23	BRCA1 (c.5339T>C, p.Leu1780Pro)	TP53 (c.916C>T, p.Arg306*)	FANCI (c.3706G>A, p.Val1236Ile)	WES	107.5	79.1
Pat24	-	TP53 (c.488A>G, p.Tyr163Cys)	-	WES	89.7	42.0
Pat25	-	TP53 (c.527G>T, p.Cys176Phe)	-	WES	107.0	49.6
Pat26	-	TP53 (c.1027G>T, p.Glu343*)	-	WES	94.2	41.1
Pat27	-	TP53 (c.742C>T, p.Arg248Trp)	ATM (c.5369A>G, p.Asp1790Glu), BRCA2 (c.6151A>G, p.Asn2051Asp), MSH6 (c.1476G>A, p.Met492Ile)	WES	85.5	41.3
Pat28	BRCA1 (c.5339T>C, p.Leu1780Pro)	TP53 (c.745A>G, p.Arg249Gly)	-	WES	93.2	57.3
Pat29	-	TP53 (c.700T>C, p.Tyr234His)	FANCG (c.944G>A, p.Ser315Asn), FANCI (c.326C>G, p.Ala109Gly)	WES	107.0	106.4
Pat30	-	TP53 (c.1035dupT, p.Glu346*)	-	WES	98.8	44.6
Pat31	-	TP53 (c.711G>C, p.Met237Ile)	-	WES	103.8	52.7
Pat32	-	-	ATM (c.1741T>G, p.Leu581Val), MLH1 (c.52C>G, p.Arg18Gly)	WES	99.9	65.4

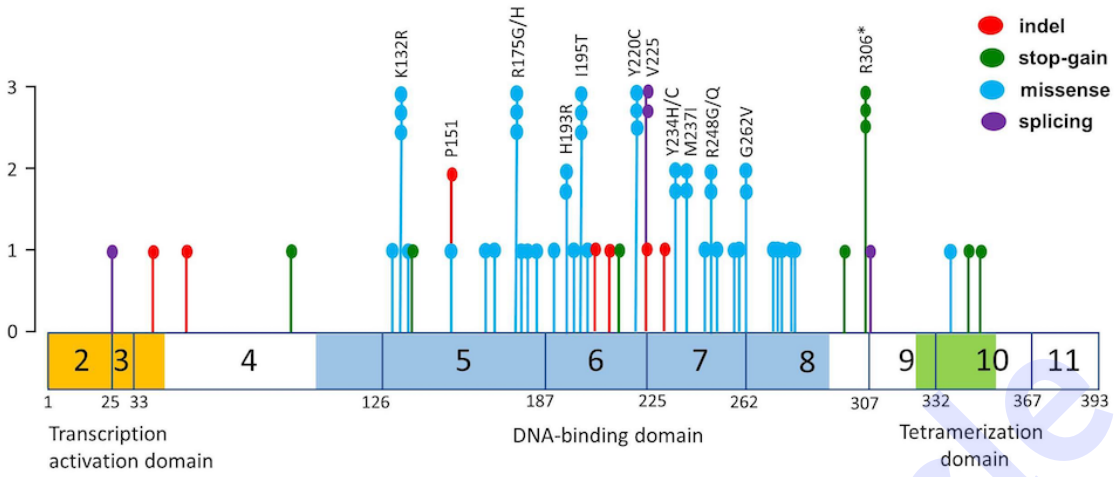
Pat33	-	TP53 (c.770T>G, p.Leu257Arg)	-	WES	97.3	53.2
Pat34	-	-	BRCA2 (c.6322C>T, p.Arg2108Cys)	WES	97.2	46.9
Pat35	-	TP53 (c.659A>G, p.Tyr220Cys)	-	WES	104.4	72.7
Pat36	BRCA1 (c.3991C>T, p.Glu1331*)	TP53 (c.273G>A, p.Trp91*)	BRCA2 (c.7633G>A, p.Val2545Ile), RAD54B (c.1721G>A, p.Gly574Glu)	WES	98.6	71.4
Pat37	-	-	-	WES	91.3	70.2
Pat38	-	TP53 (c.818G>A, p.Arg273His)	-	WES	82.4	48.2
Pat39	-	TP53 (c.830G>T, p.Cys277Phe)	-	WES	92.4	65.6
Pat40	-	TP53 (c.916C>T, p.Arg306*)	-	WES	95.8	45.9
Pat41	BRCA1 (c.5496_5506delGGTGACCCGAGinsA, p.Val1833Serfs*7)	TP53 (c.114delA, p.Ala39Glnfs*5)	BRCA2 (c.7522G>A, p.Gly2508Ser)	WES	100.1	41.3
Pat42	BRCA1 (c.1112delC, p.Pro371Leufs*3)	TP53 (c.599delA, p.Asn200Ilefs*47)		WES	89.2	48.1
Pat43	-	TP53 (c.673dupG, p.Val225Glyfs*4)	BARD1 (c.928T>C, p.Ser310Pro), FANCM (c.2240A>G, p.His747Arg)	WES	97.5	78.5
Pat44	-	TP53 (c.395A>G, p.Lys132Arg)	RAD52 (c.164G>A, p.Arg55His)	WES	96.9	51.9
Pat45	BRCA1 (c.923_924delGC, p.Ser308Lysfs*11)	TP53 (c.584T>C, p.Ile195Thr)	BRCA1 (c.922A>T, p.Ser308Cys)	WES	103.7	53.5
Pat46	BRCA1 (c.390C>A, p.Tyr130*)	-	-	WES	114.1	54.4
Pat47	BRCA1 (c.4287C>A, p.Tyr1429*)	TP53 (c.920-2A>G)	BARD1 (c.1525A>G, p.Ile509Val), FANCA (c.412A>G, p.Thr138Ala), PMS2 (c.2276-1G>C), RAD50 (c.2209C>G, p.Gln737Glu), RAD52 (c.138G>C, p.Arg46Ser)	WES	95.2	35.6

Pat48	BRCA1 (c.3442G>T, p.Glu1148*)	-	FANCE (c.1204C>T, p.Leu402Phe), PALB2 (c.718C>A, p.Pro240Thr), PALB2 (c.3054G>C, p.Glu1018Asp)	WES	97.4	68.8
Pat49	BRCA1 (c.390C>A, p.Tyr130*)	TP53 (c.711G>A, p.Met237Ile)	-	WES	100.3	49.2
Pat50	-	-	ATM (c.8246A>T, p.Lys2749Ile)	WES	94.7	56.1
Pat51	-	-	-	WES	89.0	54.2
Pat52	-	TP53 (c.538G>A, p.Glu180Lys)	FANCM (c.4378A>C, p.Ile1460Leu)	WES	100.6	38.6
Pat53	-	ATR (c.5440delA, p.Arg1814Glufs*10), MSH2 (c.387_388delTC, p.Gln130Valfs*2), RAD50 (c.2165delA, p.Lys722Argfs*14), TP53 (c.637C>T, p.Arg213*)	ATM (c.3467C>T, p.Thr1156Met), NBN (c.511A>G, p.Ile171Val), RAD50 (c.1211A>G, p.Q404R)	WES	100.2	70.9
Pat54	BRCA2 (c.2567delA, p.Asn856Ilefs*2)	TP53 (c.701A>G, p.Tyr234Cys)	FANCI (c.3907G>A, p.Glu1303Lys), SLX4 (c.5177C>T, p.Ala1726Val)	WES	106.8	58.2
Pat55	BRCA1 (c.2359delG, p.Glu787Lysfs*5)	TP53 (c.578A>G, p.His193Arg)	BRIP1 (c.613T>C, p.Phe205Leu), MSH6 (c.3111C>A, p.Phe1037Leu)	WES	101.5	53.0
Pat56	-	-	FANCM (c.2240A>G, p.His747Arg)	WES	94.7	55.4
Pat57	-	TP53 (c.587G>C, p.Arg196Pro)	-	WES	85.1	64.7
Pat58	-	TP53 (c.578A>G, p.His193Arg)	FANCG (c.8G>T, p.Arg3Leu), MSH6 (c.3772C>G, p.Gln1258Glu), NBN (c.1601A>C, p.Asn534Thr)	WES	98.6	49.5
Pat59	-	-	FANCC (c.1000C>T, p.Arg334Trp), FANCI (c.2200A>G, p.Ser734Gly)	WES	90.2	56.2

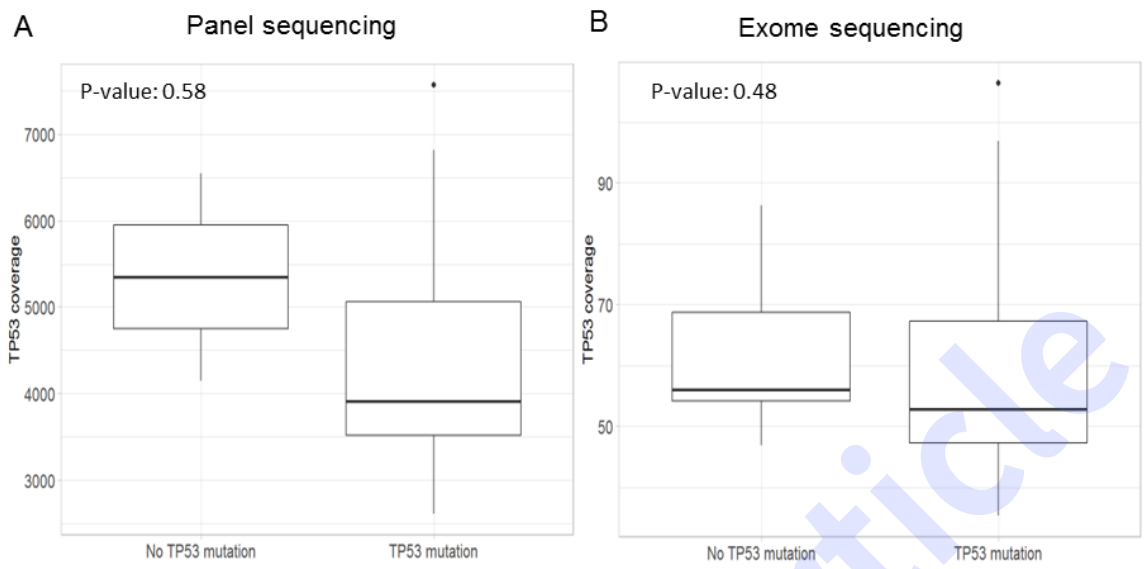
Pat60	BRCA1 (c.3020delC, p.Ser1007*)	-	ATM (c.3963G>A, p.Met1321Ile), BRCA2 (c.623T>G, p.V208G), CHEK2 (c.1486G>C, p.Ala496Pro), MSH6 (c.3245C>T, p.Pro1082Leu)	WES	122.7	81.6
Pat61	-	TP53 (c.523C>G, p.Arg175Gly)	-	WES	123.2	96.9



S4 Fig. The distribution of altered codon in *BRCA*1 and *TP53* gene without hotspot (A) The bar graph shows the number of pathogenic or likely pathogenic variants in each *TP53* codon. *TP53* R175H (5.6%) and *TP53* R248Q/W (7.9%) were observed in three (4.8%) and two (3.2%) cases among 62 patients, respectively. *TP53* K132R, I195T, Y220C, and R306* variants were observed in three patients (4.8%). (B) The bar graph shows the number of pathogenic or likely pathogenic variants in each *BRCA1* codon. *BRCA1* Y130Ter variants were observed in three patients (3/22, 13.6%).



S5 Fig. The details of the *TP53* gene mutation in this study is shown in graphic chart. *TP53* mutations were significantly enriched in DNA-binding domain.



S6 Fig. The *TP53* sequencing coverage in the two sequencing methods of panel sequencing and exome sequencing. (A) In panel sequencing, there was no statistical difference of *TP53* sequencing coverage between patients with *TP53* mutations and without it (t-test p-value=0.58). (B) In whole exome sequencing, there was also no statistical difference between patients with *TP53* mutations and without it. (t-test p-value=0.48).

S7 Table. Seven *TP53* variants with low depth (<20) in 77 patients

Pat ID	Gene	Chromosome	Position	HGVSc	HGVSp	Mutation type	mRNA	Ref	Alt	Gene ID	QUAL	Depth	Allele freq	Pathogenicity	Somatic/ Germline	methods for Somatic calls
Pat48	TP53	chr17	7579373	c.314delG	p.Gly105fs*18	Frameshift deletion	NM_000546.5	C	-	7157	255	10	0.90	Pathogenic	Somatic	Matched normal
Pat03	TP53	chr17	7579339	c.331_348delCTGGGCTTCTTGCATTCT	p.LeuGlyPheLeuHisSer111del	in-frame deletion	NM_000546.5	AGAATGCAAGAAGCCCAG	-	7157	107	12	0.33	Pathogenic	Somatic	Matched normal
Pat60	TP53	chr17	7577144	c.792_794delACT	p.Leu265del	in-frame deletion	NM_000546.5	AGT	-	7157	721	17	1.00	Pathogenic	Somatic	Matched normal
Pat34	TP53	chr17	7577129	c.809T>C	p.Phe270Ser	Missense	NM_000546.5	A	G	7157	172	9	0.67	Pathogenic	Somatic	Matched normal
Pat46	TP53	chr17	7577129	c.809T>C	p.Phe270Ser	Missense	NM_000546.5	A	G	7157	292	14	0.71	Pathogenic	Somatic	Matched normal
Pat59	TP53	chr17	7577129	c.809T>G	p.Phe270Cys	Missense	NM_000546.5	A	C	7157	83	16	0.25	Pathogenic	Somatic	Matched normal
Pat56	TP53	chr17	7577118	c.820G>T	p.Val274Phe	Missense	NM_000546.5	C	A	7157	463	19	0.89	Pathogenic	do not know	