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Original Article

BRCA1/2 mutation status in patients with metachronous breast and ovarian malignancies: clues towards the implementation of genetic counseling

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ABSTRACT

Objective: The characteristics of patients with metachronous breast and ovarian malignancies and the pathogenic role of *BRCA1/2* mutations remain poorly understood. We investigated these issues through a review of hospital records and nationwide Taiwanese registry data, followed by *BRCA1/2* mutation analysis in hospital-based cases.
Methods: We retrospectively retrieved consecutive clinical records of Taiwanese patients who presented with these malignancies to our hospital between 2001 and 2017. We also collected information from the Data Science Center of the Taiwan Cancer Registry (TCR) between 2007 and 2015. Next-generation sequencing and multiplex ligation-dependent probe amplification were used to identify *BRCA1/2* mutations and large genomic rearrangements, respectively. When *BRCA1/2* mutations were identified in index cases, pedigrees were reconstructed and genetic testing was offered to family members.
Results: A total of 12,769 patients with breast cancer and 1,537 with ovarian cancer were retrieved from our hospital records. Of them, 28 had metachronous breast and ovarian malignancies. We also identified 113 cases from the TCR dataset. Eighteen hospital-based cases underwent *BRCA1/2* sequencing and germline pathogenic mutations were detected in 7 patients (38.9%, 5 in *BRCA1* and 2 in *BRCA2*). All *BRCA1/2* mutation carriers had ovarian high-grade serous carcinomas. Of the 12 patients who were alive at the time of analysis, 5 were *BRCA1/2* mutation carriers. All of them had family members with *BRCA1/2*-associated malignancies.
Conclusions: Our results provide pilot evidence that *BRCA1/2* mutations are common in Taiwanese patients with metachronous breast and ovarian malignancies, supporting the clinical utility of genetic counseling.

Keywords: Genes, BRCA1; Genes, BRCA2; Breast Cancer; Ovarian Cancer; Metachronous Neoplasms; Genetic Counseling

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

Conceptualization: C.A., C.S.C., L.C.Y., H.K.G., L.C.H.; Data curation: L.Y.H.; Formal analysis: Y.L.Y., C.W.Y., C.P.Y., C.S.C., L.C.Y.; Funding acquisition: C.A.; Investigation: C.A., W.R.C.; Methodology: C.P.Y., C.S.C.; Resources: H.H.J., L.C.T., C.H.H., H.K.G., K.W.L., C.T.C.; Software: C.S.C.; Supervision: C.A., W.R.C., L.C.H.; Validation: C.A., L.C.Y.; Visualization: C.A., W.R.C.; Writing - original draft: C.A., L.Y.H., C.S.C.; Writing - review & editing: C.A., W.R.C., L.C.Y., L.C.H.

INTRODUCTION

Germline *BRCA1/2* mutations play a pathogenic role in most cases of hereditary breast and ovarian cancer (HBOC) syndrome [1-3]. Although *BRCA1/2* mutations increase the risk of breast and/or ovarian cancer, the tumor-specific risk figures are not equivalent and have been only partly elucidated. A combined analysis of 22 studies reported that *BRCA1* mutation carriers have a cumulative risk of breast and ovarian cancer of 65% and 39%, respectively, by 70 years of age [4]. As far as *BRCA2* mutations are concerned, the cumulative risk of breast and ovarian cancer is 45% and 11%, respectively [4]. Previous studies in both Western and Asian countries have shown that *BRCA1/2* germline mutations can be detected in approximately 12%–25% of patients with high-grade serous carcinoma of the ovary (HGSC) [5-9] whereas their prevalence is markedly lower (3.7%–4.7%) in women with breast cancer aged between 40 and 59 years [10].

In the event of metachronous malignancies, breast tumors generally precede ovarian neoplasms [11]. Notably, there is evidence that the *BRCA1/2* mutation status can influence the risk of subsequent ovarian tumors in patients with breast cancer — with 10-year risk rates of 12.7% and 6.8% for *BRCA1* and *BRCA2* mutation carriers, respectively [12]. Although published data on the risk of future breast cancer in women with an initial diagnosis of ovarian cancer remain limited [13,14], this sequence appears to be uncommon — with only 18 [13] and 12 metachronous cases [14] being reported in a 15-year cohort study.

The National Comprehensive Cancer Network guidelines recommend risk-reducing salpingo-oophorectomy (RRSO) to be performed between 35 and 45 years of age upon completion of childbearing in women harboring *BRCA1/2* mutations [15]. Despite being a risk-reducing procedure [16], RRSO remains psychologically and clinically cumbersome. For example, estrogen deprivation can lead to osteoporosis, vasomotor symptoms, and long-term complications (including cardiovascular disease and cognitive decline) [17]. Consequently, any decision to undergo RRSO should be carefully weighted in light of personal and family history data [18]. The decision to perform RRSO should be thoroughly discussed between the patient and a multidisciplinary team (consisting of gynecologic oncologists, general oncologists, genetics specialists, and pathologists) and a close follow-up schedule should be implemented.

We designed the current study with 2 main goals: 1) to shed more light on the characteristics of patients with metachronous breast and ovarian malignancies and 2) to investigate the pathogenic role of *BRCA1/2* mutations in this clinical entity. These issues were investigated through a review of hospital records and nationwide registry data, followed by *BRCA1/2* mutation analysis in hospital-based cases. In the event of *BRCA1/2* mutations being identified in the index case, pedigrees were reconstructed and genetic testing was offered to family members.

MATERIALS AND METHODS

1. Data retrieval

Ethical approval for retrospective chart review was granted by the local Institutional Review Board (approval number: 201800799B0). Owing to the retrospective nature of the study, the need for informed consent was waived. We retrospectively retrieved the clinical records of women with metachronous breast (including ductal carcinoma in situ) and ovarian malignancies who were consecutively admitted to our hospital between 2001 and 2017 (**Fig. 1**). Metachronous tumors

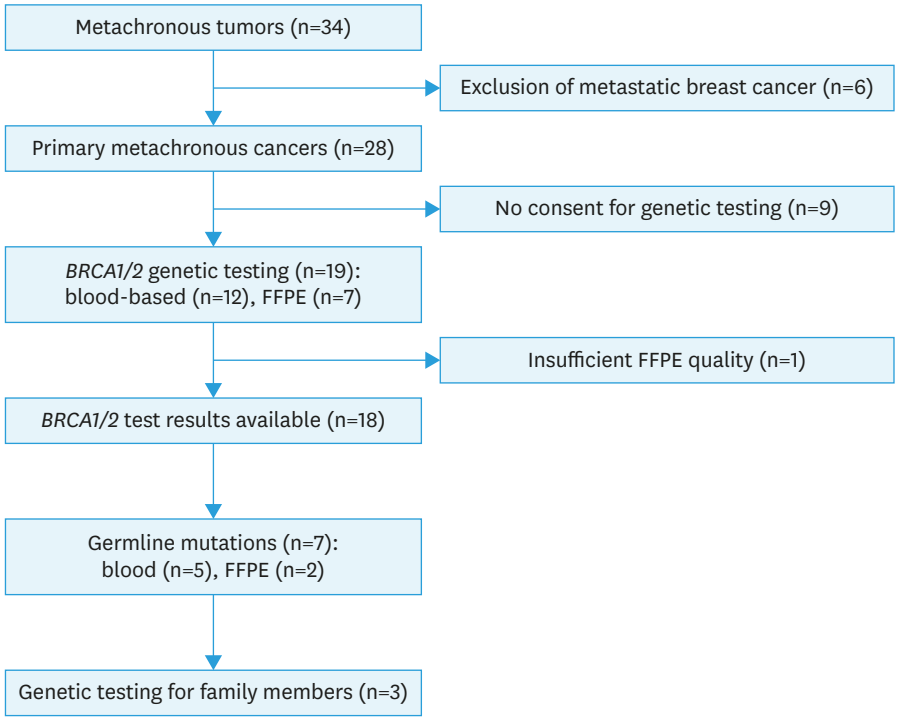

Fig. 1. Study flow chart.
FFPE, formalin-fixed paraffin-embedded.

were defined as breast or ovarian malignancies that presented more than 3 months of each other (i.e., initial diagnosis of breast cancer followed by ovarian cancer or vice versa). Besides chart review, we also collected information from the Taiwan Cancer Registry (TCR) database for the 2007–2015 period from Health and Welfare Data Science Center, Ministry of Health and Welfare. We specifically searched for the following International Classification of Diseases for Oncology, Third Edition codes: C569 (ovarian cancer) and C500–C506, C508, and C509 (breast cancer) [19]. Ovarian cancer comprised the following histological types: high- or low-grade serous carcinoma, clear cell carcinoma, endometrioid carcinoma, and mucinous carcinoma (histology codes: 8441/8461, 8460, 8310, 8380, and 8480). With regard to breast cancer, both duct (histology code: 8500) and lobular carcinoma (histology code: 8520) were included. Only female patients who received surgery as their initial treatment were deemed eligible.

2. *BRCA1/2* genetic analysis

All participants who underwent genetic analyses provided their written informed consent. DNA was extracted from peripheral blood samples (if the patient was alive) or formalin-fixed paraffin-embedded (FFPE) normal specimens (when the patient died of disease). *BRCA1/2* mutations were identified by next-generation sequencing (NGS) as previously described [20]. Multiplex ligation-dependent probe amplification (MLPA) was performed to identify large genomic rearrangements [21-23]. The SALSA MLPA P002 kit (MRC-Holland, Amsterdam, the Netherlands) was used for genomic quantification of each of the 24 *BRCA1* exons. Positive samples were reanalyzed for confirmation using the SALSA MLPA P087 kit (MRC-Holland). The SALSA MLPA P045 kit (MRC-Holland) was used for genomic quantification of the 25 *BRCA2* exons. Fragment analysis was performed on an Applied Biosystems 3500 Dx Genetic Analyzer with size standard GeneScan 500 Liz size standard (Applied Biosystems, Waltham, MA, USA). Data were interpreted using the GeneMapper software (Applied Biosystems) and

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variations in peaks areas were analyzed using the MRC Coffalyser (MRC-Holland). Sanger sequencing was used for validation purposes.

3. Library preparation and Ion S5 plus sequencing

Libraries were prepared using the Oncomine BRCA Research panel (Thermo Fisher Scientific, Waltham, MA, USA) following to the manufacturer's instructions. In brief, barcoded libraries were generated using 10 ng of DNA from each sample using an Ion AmpliSeq™ Library Kit Plus (Thermo Fisher Scientific) and an Oncomine™ BRCA research assay (Thermo Fisher Scientific). Two premixed pools of 265 primer pairs were used to generate sequencing libraries. Clonal amplification of libraries was carried out by emulsion polymerase chain reaction using an Ion Chef system (Thermo Fisher Scientific). The resulting libraries were sequenced on an Ion S5 plus Sequencer using an Ion 520 Chip and the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef (Thermo Fisher Scientific).

4. Bioinformatics analysis

Generated raw sequence data were aligned to the hg19 human reference genome using the Torrent Mapping Alignment Program implemented in the Torrent Suite software (version 5.10; Thermo Fisher Scientific). Single nucleotide variant calling was performed with the Torrent Variant Caller plug-in (version 5.10; Thermo Fisher Scientific), using the recommended somatic variant caller parameter for the BRCA Oncomine Research Panel. Candidate variants were filtered based on key parameters (strand bias, minimum allele frequency, minimal coverage, and known errorprone position). Annotation of variants was performed using the Ion Reporter Software 5.10 (Thermo Fisher Scientific Inc., Waltham, MA, USA). All relevant variants were visually inspected using the Integrative Genomics Viewer.

5. Pedigree reconstruction

In the event of *BRCA1/2* mutations being identified in the index case, pedigrees were reconstructed and genetic testing was offered to family members. To this aim, parents, siblings, and children were considered as first-degree relatives, whereas grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings were regarded as second-degree relatives.

6. Statistical analysis

Comparisons between the *BRCA1/2* wild-type (*BRCAw*) and *BRCA1/2* mutant (*BRCAm*) groups were performed using the Mann-Whitney U test for continuous variables and the Fisher's exact test for categorical variables. The comparison of the prevalence between our hospital records and TCR was performed using 2 proportion z-test. The duration of follow-up was calculated from the date of diagnosis to the date of death (or censored on the date of last follow-up). The Taiwanese National Registry of Deaths was used to confirm survival data. Cumulative survival curves were plotted with the Kaplan-Meier method and compared with the log-rank test. All calculations were performed with the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p<0.05$ (2-tailed).

RESULTS

1. Hospital chart review

A total of 12,769 patients with breast cancer and 1,537 with ovarian cancer were identified from our hospital records between 2001 and 2017 (**Fig. 1**). After the exclusion of patients with metastases, 28 cases of metachronous breast and ovarian malignancies were retrieved.

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Breast cancer followed by ovarian cancer was identified in 14 patients, whereas the remaining 14 had ovarian cancer followed by breast cancer. There were no cases of synchronous breast and ovarian cancer. The median age at diagnosis of first malignancy was 48.4 years (range: 31.6–78 years; **Table 1**). Thirteen (46.4%) patients had advanced (stage III/IV) disease. Concerning ovarian cancer histology, HGSC and CCC were identified in 50% and 25% of cases, respectively — a finding in line with the distribution observed in Taiwan for non-metachronous ovarian tumors [24].

2. TCR data

The Data Science Center of the TCR for the 2007–2015 period retrieved 7,951 and 84,904 cases of ovarian and breast cancer, respectively. Of them, 113 had synchronous/metachronous breast and ovarian malignancies. Breast cancer followed by ovarian cancer was identified in 49 patients, whereas 42 had ovarian cancer followed by breast cancer. The remaining 22 cases had synchronous breast and ovarian cancer. The median age at diagnosis of the first malignancy was 53.0 years (range: 31.0–82.0 years; **Table 1**). Fifty-eight patients (51.3%) had advanced (stage III/IV) disease. Concerning ovarian cancer histology, HGSC and CCC were identified in 56.6% and 11.3% of cases, respectively. The prevalence of metachronous ovarian and breast cancers between our hospital and TCR was not significantly different; 1.92% (17/882) versus 1.42% (113/7,959) for ovary in the hospital record and TCR, respectively for the period of 2007 and 2015 (p=0.24). Similarly, the rate of breast cancer was 0.22% (17/7,673) and 0.13% (113/84,904) for breast in the hospital record and TCR, respectively (p=0.05).

Table 1. Characteristics of patients with metachronous breast and ovarian malignancies identified in our hospital records (n=28) and the Taiwan Cancer Registry (n=113)

Characteristic	Hospital records (n=28)	Taiwan Cancer Registry (n=113)
Median age (yr)*	48.4 (31.6–78.0)	53.0 (31.0–82.0)
FIGO stage (OV)		
I–II	15 (53.6)	32 (28.3)
III–IV	13 (46.4)	58 (51.3)
Missing	0	23 (20.4)
Histology (OV)		
High-grade serous adenocarcinoma	14 (50.0)	64 (56.6)
Clear cell adenocarcinoma	7 (25.0)	13 (11.5)
Endometrioid adenocarcinoma	5 (17.9)	19 (16.8)
Mucinous adenocarcinoma	2 (7.1)	4 (3.5)
Low-grade serous adenocarcinoma	0	13 (11.5)
Differentiation (OV)		
Well-differentiated	4 (14.3)	NA
Moderately-differentiated	4 (14.3)	NA
Poorly-differentiated	20 (71.4)	NA
Receptor status (BR)		
ER– and PR–	7 (25.0)	NA
ER and/or PR+	12 (42.9)	NA
HER2+	4 (14.3)	NA
ER+, PR+, and HER2+	4 (14.3)	NA
Order of cancer diagnosis		
BR– > OV	14 (50.0)	49 (43.4)
OV– > BR	14 (50.0)	42 (37.2)
Synchronous BR/OV	0	22 (19.4)

Values are presented as number (range) or number (%).
BR, breast cancer; FIGO, International Federation of Gynecology and Obstetrics; ER, estrogen receptor; NA, not available; OV, ovarian cancer; PR, progesterone receptor.
*Age at first cancer diagnosis.

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3. *BRCA1/2* genetic analysis

Of the 28 cases of metachronous breast and ovarian malignancies identified in our study, 21 were alive and 7 died of disease. Twelve of the 21 patients (57.1%) who were alive gave their consent for genetic testing. FFPE specimens were retrieved for the 7 patients who died of disease. Because one was of insufficient quality, genetic analysis of *BRCA1/2* was conducted in a total of 18 patients (12 who were alive and 6 died of disease). Germline *BRCA1/2* mutations were identified in 7 (38.9%) cases (**Tables 2** and **3**). These mutations, including 5 in *BRCA1* and 2 in *BRCA2*, were all pathogenic variants (**Table 3**). Seven of the 8 (87.5%) cases with ovarian HGSC had germline *BRCA1/2* mutations, which were not identified in patients with other types of ovarian carcinoma. MLPA analysis did not reveal any large genomic rearrangement in the 12 patients who were alive and provided peripheral blood samples. The characteristics of patients with and without *BRCA1/2* mutations are shown in **Tables 2** and **3**. The median age at diagnosis tended to be lower in the *BRCAm* group than in the *BRCAw* group, albeit not significant. The time between breast and ovarian cancer diagnosis was longer in the *BRCAm* group than in the *BRCAw* group, although this difference did not reach statistical significance as well. The management of patients with ovarian cancer did not differ between *BRCAw* and *BRCAm* group. Patients with stage I–II disease commonly received cyclophosphamide and platinum because paclitaxel is not reimbursed by the Taiwanese National Health Insurance while paclitaxel-based chemotherapies are usually administered to patients with stage III–IV disease [24]. However, overall survival did not differ significantly in the *BRCAw* and *BRCAm* groups (**Fig. 2**). Notably, 3 of the 7 patients with *BRCAm* survived for more than 10 years.

Table 2. Characteristics of patients with metachronous breast and ovarian malignancies who underwent *BRCA1/2* testing

Characteristic	Entire cohort (n=18)	*BRCAm* (n=7)	*BRCAw* (n=11)	p-value
Median age (yr)*	47.8 (31.6–68.0)	43.9 (31.6–55.8)	48.3 (32.7–68.0)	0.439
FIGO stage (OV)				0.335
I–II	9 (50.0)	2 (28.6)	7 (63.6)	
III–IV	9 (50.0)	5 (71.4)	4 (36.4)	
Histology (OV)				<0.001
Serous adenocarcinoma	8 (44.4)	7 (100)	1 (9.1)	
Clear cell adenocarcinoma	7 (38.9)	0 (0)	7 (63.6)	
Endometrioid adenocarcinoma	3 (16.7)	0 (0)	3 (27.3)	
Mucinous adenocarcinoma	0	0	0	
Differentiation (OV)				0.685
Well-differentiated	1 (5.6)	0 (0)	1 (9.1)	
Moderately-differentiated	2 (11.1)	0 (0)	2 (18.2)	
Poorly-differentiated	15 (83.3)	7 (100)	8 (72.7)	
Receptor status (BR)				0.892
ER– and PR–	5 (29.4)	2 (28.6)	3 (30.0)	
ER and/or PR+	7 (41.2)	2 (28.6)	5 (50.0)	
HER2+	3 (17.6)	2 (28.6)	1 (10.0)	
ER+, PR+, and HER2+	2 (11.8)	1 (14.3)	1 (10.0)	
Order of cancer diagnosis				>0.999
BR– > OV	7 (38.9)	3 (42.9)	4 (36.4)	
OV– > BR	11 (61.1)	4 (57.1)	7 (63.6)	
BR– > OV (yr)	2.7 (0.6–13.4)	11.8 (1.0–13.4)	1.8 (0.6–8.0)	0.229
OV– > BR (yr)	1.8 (0.3–9.2)	2.5 (0.3–7.7)	1.8 (1.2–9.2)	0.648

Values are presented as number (range) or number (%).
BR, breast cancer; *BRCAm*, *BRCA1/2* mutant; *BRCAw*, *BRCA1/2* wild-type; FIGO, International Federation of Gynecology and Obstetrics; ER, estrogen receptor; OV, ovarian cancer; PR, progesterone receptor.
*Age at first cancer diagnosis.

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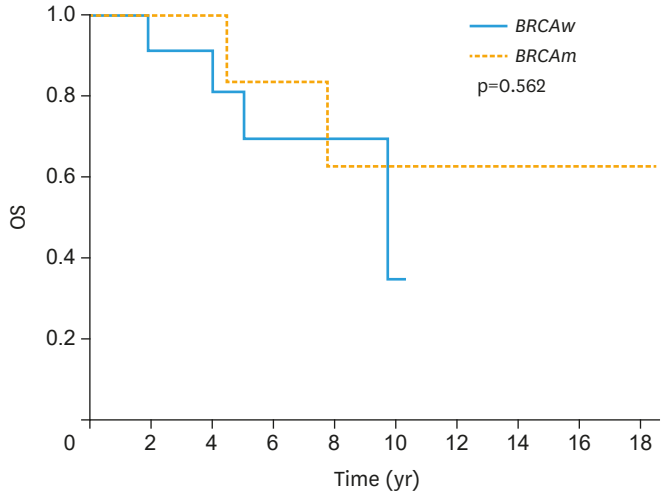
Table 3. Clinicopathological characteristics of the 7 patients with metachronous breast and ovarian malignancies harboring pathogenic *BRCA1/2* mutations

ID	Histology	Age (yr)*	FIGO stage	Grade	Gene	Exon	Nucleotide change	Amino acid change	Mutation type	FH†	FU time (yr)	Time breast to ov‡ (yr)	Breast cancer receptors		
													ER	PR	HER2
D011	Serous	32.7	3	3	*BRCA1*	1	c.5199G>A	p.Trp1733Ter	Nonsense	Breast, esophageal, stomach	14.0	11.8	–	–	–
D005	Serous	55.8	3	3	*BRCA1*	10	c.1361delG	p.Ser454fs	Frameshift	Breast, ovary	1.6	–0.3	+	+	+
D013	Serous	53.4	3	3	*BRCA2*	11	c.6484_6485delAA	p.Lys2162fs	Frameshift	Prostate, colorectal	6.6	–3.9	+	+	–
D009	Serous	41.6	2	3	*BRCA1*	10	c.928C>T	p.Gln310 Ter	Nonsense	Breast, stomach	17.4	–1.2	–	–	+
D008	Serous	43.9	1	3	*BRCA2*	11	c.5164_5165delAG	p.Ser1722fs	Frameshift	Breast, colorectal	18.5	13.4	–	–	–
D014	Serous	48.5	3	3	*BRCA1*	6	c.T303A	p.Tyr101Ter	Nonsense	NA	4.5	1.0	–	–	+
D020	Serous	39.3	3	3	*BRCA1*	10	c.3083delG	p.Arg1028Leufs*19	Frameshift	NA	7.8	–7.7	+	–	–

FIGO, International Federation of Gynecology and Obstetrics; FH, family history; FU, follow-up; ov, ovary; ER, estrogen receptor; PR, progesterone receptor; NA, not applicable.
*Age at diagnosis of the initial cancer; †Family history of cancer was considered positive when malignancies were present in first- and second-degree relatives. Parents, siblings, and children were considered as first-degree relatives, whereas grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings were regarded as second-degree relatives; ‡Time from the diagnosis of breast cancer to the occurrence of ovarian cancer.



No. at risk										
BRCAw	11	10	9	6	4	1	0	0	0	0
BRCAm	7	6	6	5	3	3	3	3	2	1

Fig. 2. OS of patients with metachronous breast and ovarian malignancies with and without *BRCA1/2* mutations. *BRCAm*, *BRCA1/2* mutant; *BRCAw*, *BRCA1/2* wild-type; OS, Overall survival.

4. Pedigree analysis

The family history of gynecology and non-gynecology malignancies was investigated in all of the 12 patients who were alive (**Supplementary Fig. 1**) and found to be positive in 11 cases. *BRCA1/2*-associated carcinomas (breast cancer, ovarian cancer, prostate cancer, and pancreatic cancer) were identified in the family members of 8 cases — including all of the 5 alive cases carrying *BRCA1/2* mutations. The distribution of tumors in family members was as follows: colorectal cancer (n=3), lung cancer (n=2), stomach cancer (n=2), esophageal cancer (n=2), and liver cancer (n=1). Of the 5 *BRCAm* patients who were alive, 3 cases had their family members tested (**Fig. 3**) [25].

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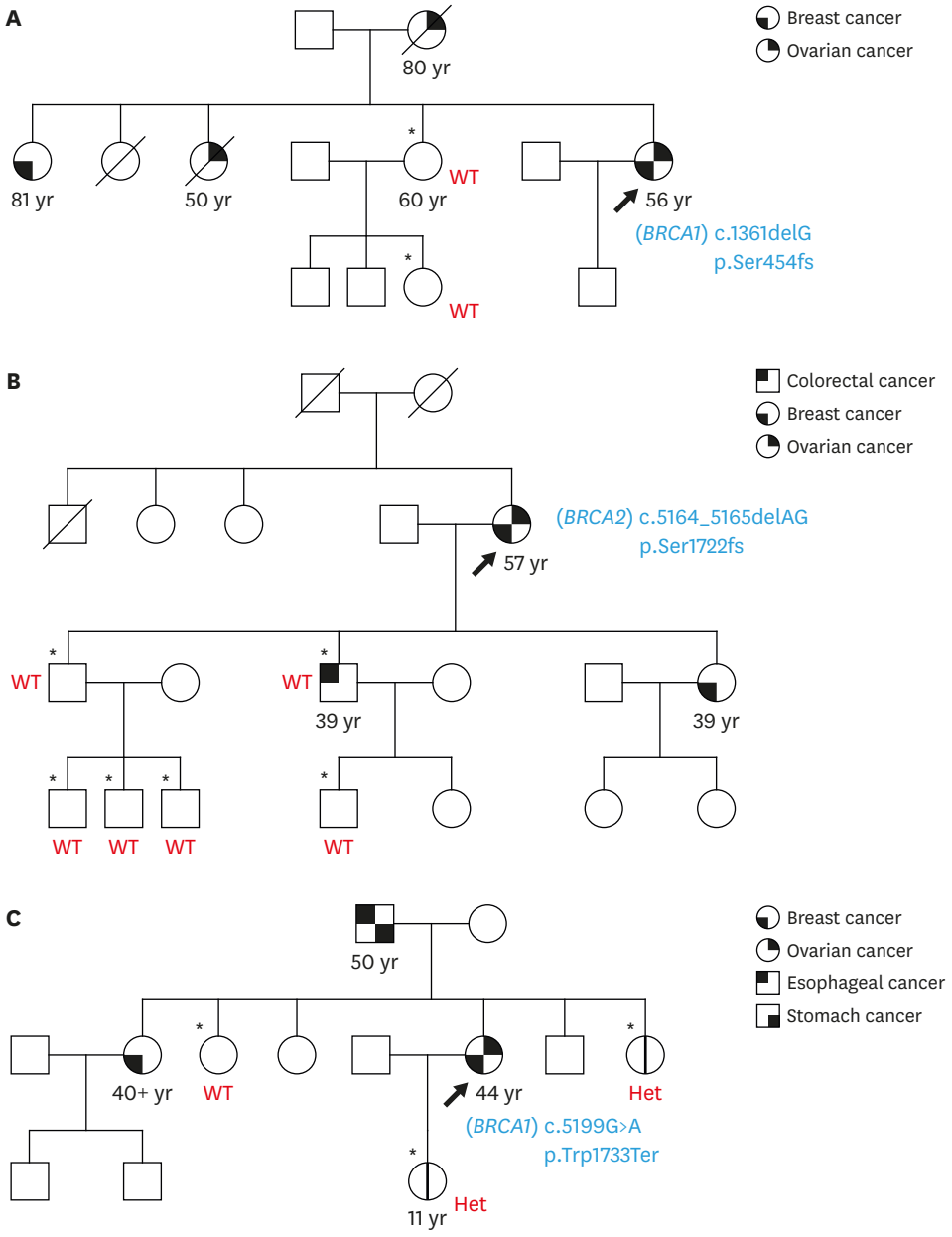

Fig. 3. Genetic testing and pedigree reconstruction of 3 index cases with metachronous breast and ovarian malignancies (A-C; arrows). The asterisk indicates the subject who underwent *BRCA1/2* gene mutation analysis. Het, heterozygous; WT, wild-type.

DISCUSSION

The main results of this study focusing on Taiwanese patients with metachronous breast and ovarian cancers can be summarized as follows. First, the prevalence of *BRCA1/2* germline mutations in this patient group was higher (38.9%) than those previously reported for patients with ovarian cancer alone (12%–25% for isolated HGSC) [5-9]. Second, all patients harboring *BRCA1/2* mutations had HGSC. Third, patients with metachronous breast and ovarian cancers commonly had a positive family history of malignancies — which included *BRCA1/2*-associated cancers in patients who tested positive for *BRCA1/2* mutations. Altogether,

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our data obtained in the Taiwanese population indicate that 1) *BRCA1/2* mutations play a pathogenic role in metachronous breast and ovarian malignancies and 2) pedigree analysis and genetic counseling are advisable in this patient group.

Previous studies have shown that the occurrence of *BRCA1/2* mutations in patients with ovarian cancer is associated with a positive family history of malignancies. For example, a Korean study reported *BRCA1/2* mutations in 61.1% and 13.5% of patients with and without a positive family history, respectively [8]. In a study from Brazil, malignancies were present in 35.5% of first and/or second-degree relatives of patients with breast and/or ovarian cancer [26]. Our current data confirm and expand these findings by showing a high prevalence of *BRCA1/2* mutations — but not of large genomic rearrangements — in Taiwanese patients with metachronous breast and ovarian malignancies. Notably, carriers of *BRCA1/2* mutations frequently showed a positive family history of *BRCA1*-(stomach, esophageal, melanoma, pancreatic malignancies) and/or *BCRA2*-associated (bile duct cancer) tumors. Our data indicate that index cases with a positive family history of cancer who did not harbor *BRCA1/2* mutations should undergo screening for multiple high- and moderate-penetrance mutations in other cancer-related genes (e.g., *RAD51C*, *RAD51D*, *ATM*, *CHEK2*, *PALB2*, *MSH6*, *MUYTH*) [27,28].

It is noteworthy that the majority (87.5%) of our patients who had metachronous breast cancer and ovarian high-grade serous carcinoma harbored *BRCA* germline mutations. We therefore believe that genetic testing and counseling should be encouraged in this patient group. On the other hand, *BRCA* germline mutations were not identified in patients with non-serous ovarian cancers (including 3 endometrioid and 7 clear cell carcinomas). Because our study cohort included a limited number of non-serous ovarian malignancies, the prevalence of *BRCA* mutations was lower in our study (42%) as compared with a previous report showing that 70% of patients with metachronous breast and ovarian cancer carried *BRCA* mutations [29]. To better guide genetic counseling practice, especially in East Asia where a larger proportion of ovarian cancers are of non-serous type, it is warranted to confirm our finding with a larger sample size, possibly from national or international consortia (e.g., Japanese HBOC consortium and Asian BRCA consortium) [30].

Despite their importance, pedigree reconstruction and genetic counseling in patients with metachronous breast and ovarian tumors continue to face significant challenges. In our study, 9 of the 21 patients (nearly half) who were alive at the time of the study declined to undergo genetic testing — which was accepted only by 3 families of the 5 cases with *BRCA1/2* mutations. Refusal of testing can be at least in part attributed to the lack of insurance coverage. However, Knerr et al. [31] reported an underutilization of *BRCA1/2* testing even in an integrated health system offering adequate coverage of genetic services. The question as to whether psychological reasons could be at work in explaining this phenomenon remains open and warrants further scrutiny. Future efforts should be tailored in improving the referral to genetic counseling and possibly offering psychological support to probands.

Our study is limited by the small sample of patients with metachronous breast and ovarian cancers, which may result in a reduced statistical power. This caveat may potentially explain the lack of significant differences between the *BRCAm* and *BRCAw* groups in terms of several clinicopathological characteristics. However, the histology of ovarian cancer was found to have significant intergroup differences, with the *BRCAm* group solely consisting of patients with high-grade serous carcinoma. Our findings need independent replication in a larger sample size to confirm their generalizability.

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These caveats notwithstanding, our study demonstrates that germline *BRCA1/2* pathogenic mutations are common among Taiwanese patients with metachronous breast cancer and ovarian high-grade serous carcinoma. Notably, *BRCA* germline mutations were absent in patients with non-serous ovarian cancers. Patients with metachronous breast cancer and ovarian high-grade serous carcinoma who are carriers of *BRCA1/2* mutations should undergo pedigree analysis and genetic counseling.

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SUPPLEMENTARY MATERIAL

Supplementary Fig. 1

Pedigrees of the 12 patients who were alive at the time of this study.

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