Acta Med. Okayama, 2022
Vol. 76, No. 4, pp. 479–483

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Case Report

Liquid Biopsy Revealed HBOC Pedigree and Led to Medical Management Among the Relatives

Chikako Ogawa[a]*, Akira Hirasawa[b], Reimi Sogawa[c], Kayoko Hasuoka[d], Shuta Tomida[e], Mashu Futagawa[c], Yusaku Urakawa[b], Mariko Kochi[c], Hideki Yamamoto[b], Keiichiro Nakamura[a], and Hisashi Masuyama[a]

Departments of [a]Obstetrics and Gynecology, [b]Clinical Genomic Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Departments of [c]Clinical Genetics and Genomic Medicine, [d]Nursing, [e]Center for Comprehensive Genomic Medicine, [e]Okayama University Hospital Biobank, Okayama University Hospital, Okayama 700-8558, Japan

A hereditary breast and ovarian cancer (HBOC) pedigree was detected via liquid biopsy, and cancer prevention was initiated for the patient's daughter, after receiving a definitive result from *BRCA* genetic testing. A 48-year-old woman with ovarian cancer was administered precision medicine, which used cell-free DNA from plasma. The results revealed a pathogenic variant of *BRCA1* as a presumed germline pathogenic mutation. We confirmed the germline pathological variant *BRCA1* c.81-1G> A and suggested treatment with a PARP inhibitor. One of her three children had the variant, was diagnosed as an unaffected pathogenic variant carrier, and was advised to initiate surveillance.

Key words: hereditary breast and ovarian cancer (HBOC), *BRCA 1*, presumed germline pathogenic variants (PGPV), germline findings, cancer precision medicine

Hereditary breast and ovarian cancer syndrome (HBOC), the leading cause of hereditary ovarian cancer, is an autosomal dominant inherited cancer susceptibility disorder caused by pathogenic germline variants in *BRCA1* or *BRCA2* (*BRCA1/2*). Ovarian, fallopian tube, and peritoneal carcinoma (OC) is the eighth most common cancer in women, and the eighth most common cause of cancer death worldwide, with over 313,900 new cases and 207,200 deaths reported annually [1,2]. Despite its low incidence, OC is the deadliest gynecological malignancy. In Japan, the age-adjusted mortality rate of ovarian cancer showed a strong increasing trend until the 1990s, and has remained high since then [3]. Although there are several causes of OC, some hereditary tumor syndromes increase the incidence of OC [4,5]. Therefore, reduction in ovarian cancer deaths is important for *BRCA1/2* pathogenic variant carriers.

Although most cases of OC are sporadic, at least 10% of patients with OC have a genetic predisposition [6,7]. The frequency of pathogenic germline variants of cancer-related genes, including *BRCA1/2*, using multigene panel testing among Japanese ovarian cancer patients, was first reported by Hirasawa *et al.* [8]. A recent large-scale multi-cancer study reported that the overall prevalence of germline *BRCA1/2* variants was 14.7% in Japanese patients with ovarian cancer [9]. The identification of these genes may provide benefits to individuals with a predisposition to OC with respect to

Received November 19, 2021; accepted March 14, 2022.
*Corresponding author. Phone: +81-86-235-7320; Fax: +81-86-225-9570
E-mail: c.fukushima@cc.okayama-u.ac.jp (C. Ogawa)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

effective prevention strategies, including risk-reductive surgery, early diagnosis, and prediction of therapeutic efficacy, such as for poly(ADP-ribose) polymerase inhibitors(PARPi). Furthermore, information and genetic diagnostic opportunities should be provided to high-risk relatives of newly diagnosed HBOC patients, as this is a key challenge that cancer genetics can help resolve.

We report the identification of an HBOC pedigree, triggered by detection of the *BRCA1* variant as a presumed germline pathogenic variant (PGPV) in circulating cell-free DNA(cfDNA) using Guardant 360 (Guardant Health, Redwood City, CA, USA). This diagnosis led to appropriate medical management for an unaffected pathogenic variant carrier in the pedigree.

Case Report

A 48-year-old woman (shown in III-3 of Fig. 1) with recurrent ovarian cancer presented to our hospital to receive precision cancer medicine. The patient had been diagnosed with stage IVB endometrioid ovarian cancer at the age of 45, and had received initial therapy including chemotherapy and surgery; however, the cancer recurred with pleural metastases and became platinum-resistant. Despite several regimen changes, the patient's condition progressed. She did not have any history of cancer, except the aforementioned ovarian cancer. In terms of family history, her paternal uncle and maternal aunt had liver cancer; however, neither breast nor ovarian cancers were noted (Fig. 1). She had three healthy children in their twenties. Her *BRCA* status was unknown, because this was before *BRCA* genetic testing was approved by insurance in Japan.

The patient and her husband visited our genetic counseling unit prior to receiving comprehensive genomic profile (CGP) testing, and we discussed the potential and importance of the germline findings. Although the patient requested a CGP using paired cancer tissue and blood tests, it was difficult to obtain a sufficient quantity and quality of DNA from a formalin-fixed paraffin-embedded (FFPE) tissue sample. We therefore suggested a liquid biopsy (with Guardant 360) using the cfDNA from her blood.

Liquid biopsy results revealed a *BRCA1* pathogenic variant (NM_007294.3(BRCA1): c. 81-1G> A) (48.1% of cfDNA) (Fig. 2). During genetic counseling, we had proposed using PARPi therapeutic agents because she was PARPi naive based on the results of the precision cancer medicine. We had informed the patient and her husband about the possibility of HBOC being a germline finding. They decided to receive PARPi treatment and to obtain a definitive diagnosis of HBOC for their relatives.

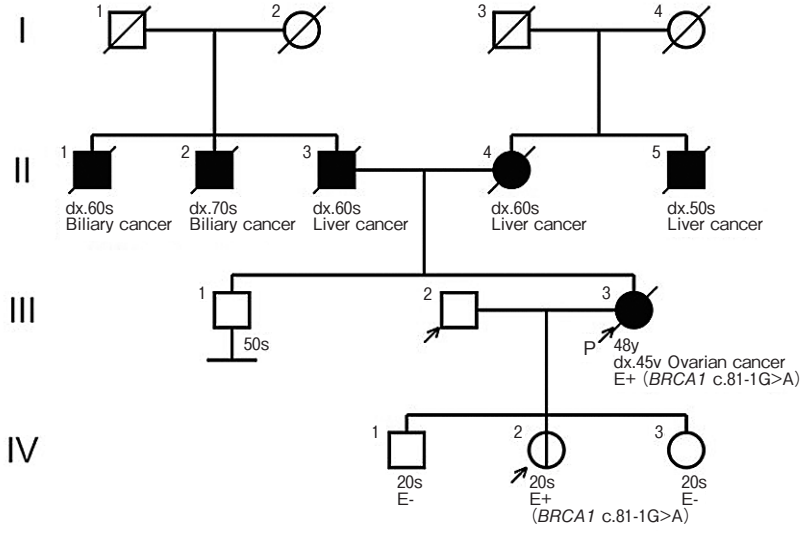

Fig. 1 Family History. The patient is shown in III-3. Her paternal uncle and maternal aunt had liver cancer, which was attributed to hepatitis B virus infection; breast, ovarian and pancreatic cancers were not noted in her pedigree. Her elder brother and three children are healthy.

Summary of Somatic Alterations & Associated Treatment Options

KEY ✓ Approved in indication ~ Approved in other indication ⊗ Lack of response

Alteration	% cfDNA or Amplification	Associated FDA-approved therapies	Clinical trial availability (see page 3)
BRCA1 Splice Site SNV	48.1%	✓ Niraparib, Olaparib, Rucaparib ~ Talazoparib	Yes
TP53 G154fs	3.5%	None	Yes

Fig. 2 Report of CGP with cfDNA. The high allele fraction of the *BRCA1* variant raised concern for a potential PGPV associated with HBOC. By contacting the testing company, the *BRCA1* splice-site SNV was disclosed as c.81-1G>A.

Before we could disclose the genetic testing results, the patient died from cancer. Her husband and three children came for the result disclosure of the genetic counseling. We informed them that the same variant was detected in the definitive diagnosis, and then provided them information about HBOC.

After genetic counseling, the three children also underwent genetic testing. The *BRCA1* variant was detected in one of the daughters (shown in IV-2 of Fig. 1). The daughter gained a good understanding of the importance of cancer prevention, including surveillance and future options for risk-reducing surgery.

We have obtained informed consent from the patient for the publication of this case.

Discussion

In recent years, CGP has been widely applied for cancer genomic medicine, and aims to identify therapeutic targets by comprehensively analyzing many cancer-related genes for mutations. In addition to the original purpose of precision cancer medicine, germline variants have been suggested. When germline variants are associated with susceptibility to cancers, hereditary cancer syndromes can be effectively diagnosed. This has significant implications for the individual, not only in terms of selecting therapies but also in preventing secondary cancers. Further, this information is useful for preventing cancers in blood relatives. The recent development of precision cancer medicine using CGP has enabled PGPV detection.

About 10% of all types of carcinomas are hereditary, and of these, HBOC is one of the most frequently observed hereditary cancer syndromes [10, 11]. The most frequently identified PGPVs are the *BRCA1/2*, which are mostly observed in the germline [13, 14]. There are ethnic differences in the prevalence of *BRCA* variants. In Japan, in a study of hereditary breast cancer conducted by Momozawa *et al.* [12], *BRCA1/2* was identified in 0.21% of 11,241 Japanese female controls. In ovarian cancer, the CHARLOTTE study [9] found *BRCA1/2* variants in 14.7% of all ovarian cancers, and in 30% of high-grade serous cancers. The *BRCA1* pathogenic variant was initially detected as a PGPV in the patient. The diagnosis of HBOC is important for cancer prevention in patients and their families, and OC patients should be actively tested. Our department has performed BRCA1/2 genetic testing for essentially all epithelial OC patients since the Japanese regulatory agency approved BRCA1/2 genetic testing for such patients under the national health insurance in 2020.

When tumor DNA cannot be obtained from FFPE, as in this case, liquid biopsy becomes a good alternative. In addition, liquid biopsy provides information on the frequency of clones and changes in drug resistance during treatment [15]. Circulating cell-free DNA (cfDNA) is released from healthy and diseased cells, mainly by rupture, necrosis, or apoptosis. A few studies have evaluated germline cancer predisposition by cfDNA [16, 17]. Salvin *et al.* [18] noted that in a study of 10,888 patients with advanced solid cancers (including 210 OC patients) who underwent cell-free circulating tumor DNA sequencing, PGPV was found in 1.5%, and in 8.4% of OC, the highest of all cancers. They also reported that PGPV was found more frequently in patients younger than 50 years. This indicates that cfDNA sequencing can inform patients of the possibility of incidental germline findings, and a counseling sys-

tem for hereditary tumors should be in place. Nevertheless, the evaluation of cfDNA germline sequences cannot replace validated hereditary cancer genetic testing. This case can be regarded as a model in which CGP by cfDNA sequencing and genetic counseling were seamlessly linked.

The present case also raised another issue—the approach taken to educate relatives living far away from the patient. In this case, the patient's husband was key, and his understanding of hereditary cancer was effective in alleviating the psychological burden on the patient and her children, thus encouraging them to decide on genetic testing and continuing medical management. While it made good sense for blood relatives to be gathered for genetic counseling, it was difficult in practice. Better access to genetic counseling is crucial, not only for family members, but also for patients who may not have access to appropriate genetic counseling facilities.

In conclusion, this case reveals not only PGPV from cfDNA-based liquid biopsy as precision medicine, but also relays the process of ensuring definitive diagnosis and medical management. Since any genetic panel testing has the potential to reveal PGPV, a seamless link between precision medicine and genetic counseling is important, and can provide medical management to families with the HBOC pedigree. To manage HBOC families, including relatives, it is important to strengthen the establishment of a nationwide system of HBOC medical care in Japan.

Acknowledgments. This research was partly supported by a Health Labour Sciences Research Grant (20EA1027).

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global Cnacer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin (2021) 71: 209–249.
2. Zheng L, Cui C, Shi O, Lu X, Li YK, Wang W, Li Y and Wang Q: Incidence and mortality of ovarian cancer at the global, regional, and national levels, 1990–2017. Gynecol Oncol (2020) 159: 239–247.
3. Katanoda K, Hori M, Saito E, Shibata A, Ito Y, Minami T, Ikeda S, Suzuki T and Matsuda T: Updated Trends in Cancer in Japan: Incidence in 1985-2015 and Mortality in 1958-2018-A Sign of Decrease in Cancer Incidence. J Epidemiol (2021) 31: 426–450.
4. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, Jervis S, van Leeuwen FE, Milne RL, Andrieu N, Goldgar DE, Terry MB, Rookus MA, Easton DF, Antoniou AC; BRCA1 and BRCA2 Cohort Consortium, McGuffog L, Evans DG, Barrowdale D, Frost D, Adlard J, Ong KR, Izatt L, Tischkowitz M, Eeles R, Davidson R, Hodgson S, Ellis S, Nogues C, Lasset C, Stoppa-Lyonnet D, Fricker JP, Faivre L, Berthet P, Hooning MJ, van der Kolk LE, Kets CM, Adank MA, John EM, Chung WK, Andrulis IL, Southey M, Daly MB, Buys SS, Osorio A, Engel C, Kast K, Schmutzler RK, Caldes T, Jakubowska A, Simard J, Friedlander ML, McLachlan SA, Machackova E, Foretova L, Tan YY, Singer CF, Olah E, Gerdes AM, Arver B and Olsson H: Risks of Breast, Ovarian, and contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA (2017) 317: 2042–2416.
5. Bonadona V, Bonaïti B, Olschwang S, Grandjouan S, Huiart L, Longy M, Guimbaud R, Buecher B, Bignon YJ, Caron O, Colas C, Noguès C, Lejeune-Dumoulin S, Olivier-Faivre L, Polycarpe-Osaer F, Nguyen TD, Desseigne F, Saurin JC, Berthet P, Leroux D, Duffour J, Manouvrier S, Frébourg T, Sobol H, Lasset C and Bonaïti-Pellié C: Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA (2011) 305: 2304–2310.
6. Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, Bernards SS, Casadei S, Yi Q, Burger RA, Chan JK, Davidson SA, Mannel RS, DiSilvestro PA, Lankes HA, Ramirez NC, King MC, Swisher EM and Birrer MJ: Inherited Mutations in Women With Ovarian Carcinoma. JAMA Oncol (2016) 2: 482–490.
7. Kanchi KL, Johnson KJ, Lu C, McLellan MD, Leiserson MD, Wendl MC, Zhang Q, Koboldt DC, Xie M, Kandoth C, McMichael JF, Wyczalkowski MA, Larson DE, Schmidt HK, Miller CA, Fulton RS, Spellman PT, Mardis ER, Druley TE, Graubert TA, Goodfellow PJ, Raphael BL, Wilson RK and Ding L: Integrated analysis of germline and somatic variants in ovarian cancer. Nat Commun (2014) 5: 3156.
8. Hirasawa A, Imoto I, Naruto T, Akahane T, Yamagami W, Nomura H, Masuda K, Susumu N, Tsuda H and Aoki D: Prevalence of pathogenic germline variants detected by multigene sequencing in unselected Japanese patients with ovarian cancer. Oncotarget (2017) 8: 112258–112267.
9. Enomoto T, Aoki D, Hattori K, Jinushi M, Kigawa J, Takeshima N, Tsuda H, Watanabe Y, Yoshihara K and Sugiyama T: The first Japanese nationwide multicenter study of BRCA mutation testing in ovarian cancer: CHARaterizing the cross-sectionaL approach to Ovarian cancer genetic Testing of BRCA (CHARLOTTE). Int J Gynecol Cancer (2019) 29: 1043–1049.
10. Schrader KA. Cheng DT, Joseph V, Prasad M, Walsh M, Zehir A, Ni A, Thomas T, Benayed R, Ashraf A, Lincoln A, Arcila M, Stadler Z, Solit S, Hyman DM, Zang L, Klimstra D, Ladanyi M, Offit K, Berger M and Robson M: Germline Variants in Targeted Tumor Sequencing Usin Matched Normal DNA. JAMA Oncol (2016) 2: 104–111.
11. Neben CL. Zimmer AD, Stedden W, van den Akker J, O'Connor R, Chan RC, Chen E, Tan Z, Leon A, Ji J, Topper S and Zhou AY: Multi-Gene Pane Testing of 23,179 Individuals for Hereditary Cancer Risk Identifies Pathogenic Variant Carriers Missed by Current Genetic Testing Guidelines. J Mol Diagn (2019) 21: 646–657.
12. Momozawa Y, Iwasaki Y, Parsons MT, Kamatani Y, Takahashi A, Tamura C, Katagiri T, Yoshida T, Nakamura S, Sugano K, Miki Y, Hirata M, Matsuda K, Spurdle AB and Kubo M: Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. Nat Commun (2018) 9: 4083.
13. Schrader KA, Cheng DT, Joseph V, Prasad M, Walsh M, Zehir A, Ni A, Thomas T, Benayed R, Ashraf A, Lincoln A, Arcila M,

Stadler Z, Solit D, Hyman DM, Zang L, Klinmstra D, Ladanyi M, Offit K, Berger M and Robson M: Germline Variants in Targeted Tumor Sequencing Using Matches Normal DNA. JAMA Oncol (2016) 2: 104–111.
14. Meric-Bernstam F, Brusco L, Danieles M, Wathoo C, Bailey AM, Strong L, Shaw K, Lu K, Qi Y, Zhao H, Lara-Guerra H, Litton J, Arun B, Eterovic AK, Aytac U, Routbort M, Subbiah V, Janku F, Davies MA, Kopetz S, Mendelsohn J, Mills GB and Chen K: Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic prfiling protocol. Ann Oncol (2016) 27: 795–800.
15. Lin KK, Harrell MI, Oza AM, Oaknin A, Ray-Coquard I, Tinker AV, Helman E, Radke MR, Say C, Vo LT, Mann E, Isaacson JD, Maloney L, O'Malley DM, Chambers SK, Kaufmann SH, Scott CL, Konecny GE, Coleman RL, Sun JX, Giordano H, Brenton JD, Harding TC, McNeish IA and Swisher EM: BRCA Reversion Mutations in Circulating Tumor DNA Predict Primary and Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma. Cancer Discov (2019) 9: 210–219.
16. Hu Y, Alden RS, Odegaard JI, Fairclough SR, Chen R, Heng J, Feeney N, Nagy RJ, Shah J, Ulrich B, Gutierrez M, Lanman RB, Garber JE, Paweletz CP and Oxnard GR: Discrimination of germline EGFR T790M mutations in plasma cell-free DNA allows study of prevalence across 31,414 cancer patients. Clin Cancer Res (2017) 23: 7351–7359.
17. Ratajska M, Koczkowska M, Źuk M, Gorczynski A, Kuzniacka A, Stukan M, Biernat W, Limon J and Wasag B: Detection of BRCA1/2 mutations in circulating tumor DNA from patients with ovarian cancer. Oncotarget (2018) 8: 101325–101332.
18. Slavin TP, Banks KC, Chudova D, Oxnard GR, Odegaard JI, Nagy RJ, Tsang KWK, Neuhausen SL, Gray SW, Cristofanilli M, Rodriguez AA, Bardia A, Leyland-Jones B, Janicek MF, Lilly M, Sonpavde G, Lee CE, Lanman RB, Meric-Bernstam F, Kurzrock R and Weitzel JN: Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing. J Clin Oncol (2018) 36: 3459–3465.