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Research Article

BRCA Tumor Analysis as Molecular Screening for Germline Testing

Alejandro González Morales^{1#}, Enrique Lastra Aras^{1#}, Mercedes Durán Domínguez^{3#}, Patricia Saiz López², M^a del Mar Infante Sanz³, Laura Ortega Morán⁴, Iria Gallego Gallego⁵, Gonzalo García González⁵, Guillermo Crespo Herrero¹, Blanca Ascensión Hernando Fernández-Aranguiz¹ and Cooperative group: Burgos-Valladolid-Madrid*

¹Medical Oncology Service, University Hospital of Burgos, Spain

²Anatomical Pathology Service, University Hospital of Burgos, Spain

³Institute of Genetics and Molecular Biology, University of Valladolid, Spain

⁴Medical Oncology Service, Gregorio Marañón General University Hospital, Spain

⁵Medical Oncology Service, University Hospital of Móstoles, Spain

#Equal contributors and first co-authors

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ABSTRACT

Background: In patients with advanced high-grade serous ovarian cancer (HGOC) and prostate adenocarcinoma, the identification of somatic/germline BRCA1/2 mutations allows new therapeutic opportunities. To estimate the prevalence of somatic and germline BRCA1/2 mutations in non-mucinous high grade ovarian/fallopian tube/peritoneal extraovarian cancer (NMHGOC) and prostate adenocarcinoma. **Methods:** Prevalence was established by analyzing patients with NMHGOC or prostate adenocarcinoma, with a BRCA1/2 study in the tumor between 2017 and 2018. Whether a germline study had been carried out was subsequently reviewed.

Results: 10 patients out of 43 (23.3%) with NMHGOC had a BRCA1/2 mutation in the tumor. 9 patients (20.9%) presented a BRCA1/2 mutation in the germline setting (2 without tumor result due to limited tissue sample). 3 patients (6.9%) had only somatic mutations. 30% of the mutations in the tumor were, therefore, somatic mutations. Of the 9 patients with prostate adenocarcinoma, 2 (22.2%) had a BRCA2 mutation in the tumor. While 1 (11.1%) had the mutation in the germline setting, 1 patient (11.1%) had only somatic mutations.

Conclusion: In our series, the prevalence of somatic and germline BRCA1/2 mutations in NMHGOC is similar to that reported in the literature. Whereas somatic mutations are only present at the neoplastic tissue, the rate of mutations in the tumor is higher than in the germline setting. A more effective diagnostic and predictive strategy could be achieved with tumor BRCA analysis as the first attempt. Initial results in prostate adenocarcinoma point to the same conclusion for this tumor.

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Introduction

The selection clinical criteria to study hereditary cancer syndromes is cumbersome and can miss diagnoses due to the incomplete penetrance and variable phenotype of susceptibility germline mutations. A universal

diagnostic strategy aims to avoid these criteria and looks to improve diagnostic sensitivity among the general population with cancer.

Beyond its potential as a biomarker for treatment, the BRCA germline mutation prevalence of 22.6% in Australian population-based high-

*Correspondence to: Mercedes Durán Domínguez, Ph.D., Institute of Genetics and Molecular Biology, University of Valladolid, IBGM, Lab-B-7. C/Sanz y Forés, 3, 47003 Valladolid, Spain; Tel: +34983184809; Fax: +34983184800; E-mail: merche@ibgm.uva.es

grade serous ovarian cancer (HGSOC), endorsed by a meta-analysis with a prevalence of 14.5%, has been acknowledged and a universal strategy for hereditary breast and ovarian cancer (HBOC) syndrome diagnosis in these tumors has been adopted, although without cost-effectiveness comparative studies [1, 2]. In unselected metastatic prostate cancer series, the assessment of 20 genes involved in DNA integrity maintenance provide a prevalence of 11.8% germline mutation carriers (6.2% for BRCA mutations), which could shortly be below 10% in the population without clinical criteria [3]. In the Spanish population of unselected metastatic castration resistant prostate cancer (mCRPC), the prevalence of germline mutations is 16.2% (4.3% for BRCA mutations) with a panel of 107 genes [4].

These figures could warrant a universal strategy for hereditary syndrome diagnosis in metastatic prostate cancer patients. However, without cost-effectiveness studies, with the high prevalence of metastatic prostate cancer and the law for germline testing in Spain, even knowing the efficacy of poly ADP-ribose polymerase PARP inhibitors in homologous recombination repair defective (HRRD) metastatic prostate cancers; it is not likely to achieve a positive balance for the establishment of such a universal screening in our country [5, 6]. The complexity of a pre-test counseling is also an issue when dealing with a germline multiple gene panel.

With a 410-gene tumor assay MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets) and a paired germline set of 76 genes in histology-agnostic cancer patients, of the 103 pathogenic BRCA mutations, 59 were germline in origin, whereas 44 were detected only in the tumor [7]. Tumor analyses detect more mutations in hereditary cancer predisposition genes than germline tests and this has been clearly stated for HGSOC in different studies: the results reported by The Cancer Genome Atlas (TCGA), the analysis of Homologous Recombination (HR) genes, the review of several series and a retrospective study of a randomized phase II trial [8-11]. In the daily practice of a tertiary hospital, we estimated the prevalence of somatic and germline BRCA1/2 mutations in non-mucinous high grade ovarian/fallopian tube/peritoneal extraovarian cancer (NMHGOC) and prostate adenocarcinoma for a different universal predictive and diagnostic strategy.

Methods

I Trial Design and Patients

We designed a retrospective study of prospective cohorts of patients with NMHGOC and prostate adenocarcinoma, unselected for family history, consecutively assisted at a medical oncology service of a tertiary hospital, and who in their standard clinical management have followed BRCA1/2 tumor testing for prognostic, predictive or diagnostic objectives, before a germline analysis.

II End Points

The objective was to estimate the prevalence of BRCA1/2 germline mutations (mutations in the lineage of germ cells that are transmitted to offspring) and the prevalence of BRCA1/2 somatic mutations (mutations

that occur in DNA after conception), in prospective cohorts of NMHGOC patients and prostate adenocarcinoma patients.

III Assessments

In daily practice, some assisted NMHGOC patients followed a tumor BRCA1/2 mutation study, after DNA extraction, with Next Generation Sequencing (NGS) Illumina (MiSeq); libraries generated with the kit BRCA MASTR Plus Dx (Mutiplicon); bioinformatic analysis performed with Sophia Genetics platform designed for detection of SNPs and Indels, but unable to detect CNVs; minimum coverage 3475X. The analysis includes complete coding sequences of both genes and adjacent intronic sequences.

In daily practice, some assisted patients with prostate adenocarcinoma followed a tumor BRCA1/2 mutation study, after DNA extraction, with the OncoPrint™ BRCA Research Assay of two pools of AmpliSeq™ oligonucleotide primers and associated reagents to generate amplicon libraries for next-generation sequencing (NGS) on Ion Torrent™ platforms. The platform is endowed for highly uniform coverage across all coding exons and splice sites for efficient sequencing and accurate analysis. The OncoPrint BRCA Assay is a complete kit facilitating the amplification of the entire exonic region of both BRCA genes from FFPE tissue. The assay is aligned with bioinformatic workflows within Torrent Suite™ and Ion Reporter™ analysis software that utilize optimized variant calling parameters for SNV, InDel, and large exon/gene deletion/duplication detection.

Variants found were described following HGVS guidelines, and classified according to different databases: Mutation of BRCA1 and BRCA2 sequence variants that have been clinically reclassified using a quantitative integrated evaluation (Link), BRCA Share (Link1, Link2), ClinVar (Link), BRCA Exchange (Link), A Fanconi Anemia Mutation database (Link), BRCA1 CIRCOS (Link), 1000 genomes Project (Link) and Exome Sequencing Project (ESP) (Link). Information is interpreted under standardized criteria developed by ENIGMA (Link).

Following an established universal diagnostic strategy in HBOC, every patient with NMHGOC was to be assisted at a Genetic Counseling Unit (GCU). All the patients with a BRCA tumor pathogenic variant were sent to a GCU, for a genetic counseling process in accordance with Spanish law [5]. They signed an Informed Consent for germline testing of, at least, the deleterious mutation uncovered at the tumor. The pathogenic variants were confirmed or discarded at the germline setting by Sanger sequencing, HA_CAE (heteroduplex in capillary electrophoresis) and Big Dye Terminators sequencing in an ABI 3100 analyzer.

IV Trial Oversight

The trial was designed and performed by medical oncologists at a tertiary hospital. The protocol of the present study was developed in accordance with the Declaration of Helsinki, with Good Clinical Practice as defined by the International Conference on Harmonization, CPMP/ICH/135/95, and approved by the Committee for Ethics and Clinical Trials of the University Hospital of Burgos. Signed consent was waived for the study, since it was a retrospective analysis, reviewing standard clinical practice,

developed in the best care of the patients' health, without harm for the participating subjects and with honest management of the linked and created data.

V Statistical Analysis

Estimation of mutation prevalence (somatic/germline) was calculated as the percentage of patients with a gene mutation (somatic/germline) out of the total number of patients in the cohort. Comparisons between the prevalences of this study and those reported in the literature were merely descriptive.

Results

In our hospital, between June 2017 and September 2018, BRCA1/2 genes were studied in the tumors of 43 patients diagnosed with ovarian carcinoma (38 cases, 88.4%), extra-ovarian peritoneal carcinoma (2 cases, 4.7%), fallopian tube carcinoma (1 case, 2.3%), synchronous ovarian and endometrial carcinoma (1 case, 2.3%) and ovarian carcinoma/extra-ovarian peritoneal carcinoma (1 case, 2.3%) (Table 1).

Table 1: Patients with ovarian carcinoma, extra-ovarian peritoneal carcinoma and fallopian tube carcinoma with BRCA1/2 study.

Patient	Primary tumor	Tumor BRCA 1/2	GCU visit	Germline BRCA 1/2
1	Ovarian	NPV	No	NS
2	Ovarian	NPV	Yes	NPV
3	Ovarian	NPV	Yes	NPV
4	Synchronous ovarian and endometrial cancer	NPV	No	NS
5	Ovarian	NPV	Yes	NPV
6	Ovarian	NPV	Yes	NPV
7	Ovarian	BRCA1 mutation	Yes	Normal
8	Ovarian	BRCA1 mutation	Yes	BRCA1 mutation
9	Ovarian	NPV	Yes	NPV
10	Ovarian	NPV	Yes	NS
11	Ovarian	NPV	No	NS
12	Ovarian	BRCA1 mutation	Yes	Normal
13	Ovarian	NPV	No	NS
14	Ovarian	BRCA1 mutation	Yes	BRCA1 mutation
15	Ovarian	BRCA2 mutation	Yes	BRCA2 mutation
16	Ovarian	NPV	Yes	NS
17	Ovarian	NPV	Yes	NS
18	Ovarian	BRCA1 mutation	Yes	BRCA1 mutation
19	Ovarian	NPV	Yes	NPV
20	Ovarian	NPV	No	NS
21	Ovarian	NPV	Yes	NPV
22	Ovarian	Insufficient sample	Yes	NPV
23	Ovarian	NPV	Yes	NS
24	Ovarian	NPV	Yes	NPV
25	Peritoneal extraovarian carcinoma	NPV	No	NS
26	Ovarian	NPV (BRCA2 VUS)	Yes	NPV
27	Ovarian	NPV	Yes	NPV
28	Ovarian	NPV	Yes	NS
29	Ovarian	Insufficient sample	Yes	BRCA1 mutation
30	Ovarian	NPV	No	NS
31	Peritoneal extraovarian carcinoma	NPV	Yes	NPV
32	Ovarian/peritoneal extraovarian carcinoma	NPV	No	NS
33	Ovarian	NPV	No	NS
34	Ovarian	NPV	Yes	NPV
35	Ovarian	NPV	Yes	NPV (BRCA1 VUS)
36	Ovarian	NPV	Yes	NPV
37	Ovarian	Insufficient sample	Yes	BRCA2 mutation

38	Ovarian	BRCA1 mutation	Yes	Normal
39	Ovarian	BRCA1 mutation	Yes	BRCA1 mutation
40	Ovarian	BRCA1 mutation	Yes	BRCA1 mutation
41	Ovarian	Insufficient sample	No	NS
42	Fallopian tube	BRCA2 mutation	Yes	BRCA2 mutation
43	Ovarian	NPV	No	NS

NPV: no pathogenic variants (when both genes are studied); Normal: no mutation (when only the pathogenic variant uncovered at the tumor is studied); NS: not studied; VUS: variant of unknown significance.

We found 28 patients (65.1%) with non-pathogenic variants, 10 patients (23.3%) with a BRCA1/2 mutation (8 patients with a BRCA1 mutation, 2 with a BRCA2 mutation), 4 patients (9.3%) with insufficient sample to perform the study and 1 patient (2.3%) with a BRCA2 variant of uncertain significance.

Of the 43 patients, 32 (74.1%) were assisted at our hospital's GCU, and a BRCA1/2 germline test was performed in 27 due to a family history of cancer, early age at diagnosis of cancer, insufficient tumor sample for BRCA study, or BRCA1/2 mutations in the tumor. We found BRCA1/2 germline mutations in 9 patients (20.9% of the 43 patients). In the 24 patients who completed the BRCA1/2 analysis in the germline setting and in the neoplastic tissue, the tumor mutation rate was 41.7% (10/24) and the germline prevalence of mutations was 29.2% (7/24). None of the

patients without a BRCA1/2 mutation in the tumor had a germline mutation.

All the patients with a BRCA1/2 mutation in the tumor (10 patients) were studied at the germline setting, finding the same mutation in 7 patients. One of the 3 somatic mutations presented an allelic fraction below 50% which, in the context of a good tumor purity and quantity, could have raised the suspicion of its absence as a hereditary variation. We found 2 patients with a BRCA germline mutation whose tumors were not studied due to insufficient sample (one with a BRCA1 germline mutation and another with a BRCA2 germline mutation).

Therefore, in the cohort of patients with NMHGOC, the estimated prevalence of BRCA1/2 somatic mutations is 6.9%, the estimated prevalence of germline mutations being 20.9% for a BRCA1/2 tumor mutation rate of 23.3% (Figure 1).

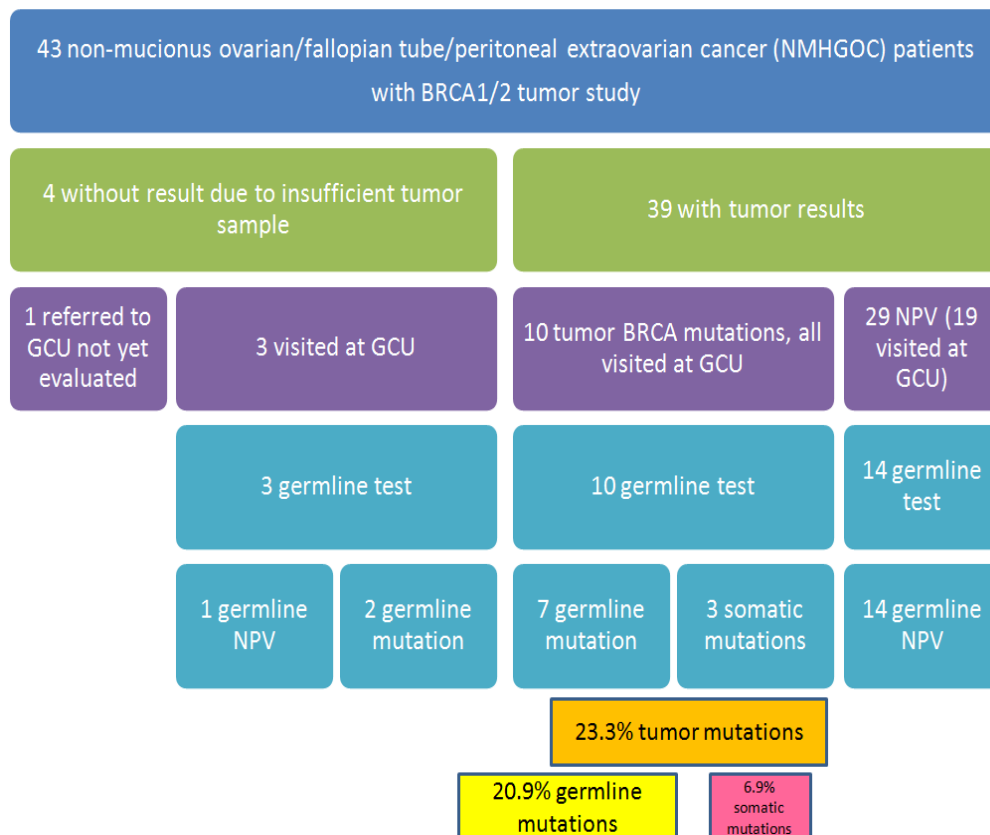


Figure 1: Development and results of the study (cohort of NMHGOC: non-mucinous high grade ovarian/fallopian tube/peritoneal extraovarian cancer). GCU: Genetic Counseling Unit; NPV: no pathogenic variants.

In patients with prostate adenocarcinoma, one patient was analyzed in 2017 and 8 in 2018. 2 patients (22.2%) had a BRCA2 mutation in the

tumor. One was studied in the germline setting without the mutation, so he had a somatic mutation. The other patient could not be studied but his daughter had the mutation in the germline setting, so this patient was an

obligatory carrier. Therefore, in the cohort of patients with prostate adenocarcinoma, the estimated prevalence of somatic mutations is

11.1%, the estimated prevalence of germline mutations is 11.1% for a BRCA1/2 tumor mutation rate of 22.2% (Table 2).

Table 2: Patients with prostate adenocarcinoma and BRCA1/2 study.

Patient	Year of the study	Tumor	Tumor BRCA 1/2	Germline BRCA 1/2
1	2017	Prostate adenocarcinoma	NPV	NS
2	2018	Prostate adenocarcinoma	NPV	NS
3	2018	Prostate adenocarcinoma	NPV	NS
4	2018	Prostate adenocarcinoma	NPV	NS
5	2018	Prostate adenocarcinoma	BRCA2 mutation	Normal
6	2018	Prostate adenocarcinoma	BRCA2 mutation	BRCA2 mutation
7	2018	Prostate adenocarcinoma	NPV	NS
8	2018	Prostate adenocarcinoma	NPV	NS
9	2018	Prostate adenocarcinoma	NPV	NS

NPV: no pathogenic variants (when both genes are studied); Normal: no mutation (when only the pathogenic variant uncovered at the tumor is studied); NS: not studied.

Discussion

In our hospital, the estimated prevalence of BRCA1/2 mutations in tumors of patients with NMHGOC is 23.3%, while the estimated prevalence of germline mutations for the same population is 20.9%, in concordance with the published literature [1, 8]. In the 24 patients who completed both BRCA1/2 analyses, in the germline setting and in the neoplastic tissue, the tumor mutation rate was 41.7% (10/24) and the germline prevalence of mutations was 29.2% (7/24). We consider that the analysis in the tumor brought to light 6.9% of patients with somatic mutations, which would not be detected with a germline analysis.

We have also estimated the prevalence of BRCA1/2 mutations in 9 patients with prostate adenocarcinoma not selected by family history. We found tumor mutations in 2 (22.2%), with one somatic mutation (11.1%) and one germline mutation (11.1%), a figure higher than those described by Pritchard *et al.* and Castro *et al.* although with a low number of cases analyzed [3, 4].

The technology for ovarian tumor BRCA testing in our study is not provided with a bioinformatic tool for the detection of CNV, and some large rearrangements in BRCA could have been missed. The estimated prevalence of pathogenic somatic and germline BRCA mutations could have been affected to a low extent, because the prevalence of somatic BRCA large rearrangements has been reported to be <1.75% in a retrospective analysis of 114 ovarian cancers studied (prevalence of germline large rearrangements <2.6% in this study), and the germline prevalence of large BRCA1/2 rearrangements in the biggest cohort of patients studied (including ovarian cancer patients) does not exceed 3% [11, 12].

Not all the 43 patients with NMHGOC have been tested for BRCA1/2 mutations at the germline setting, but every mutation in the tumor was studied to dismiss hereditary predisposition. It would be rare that a BRCA germline mutation (different to a large rearrangement) could have remained undetected at the tissue with a good tumor purity and quantity. This could have happened with bad tissue preservation, lack of sample, or low-quality DNA extraction. When any of these events were reported, as happened with four patients, the tumor test was not pursued and the

germline analysis was established as a better choice, to be done in a universal diagnostic strategy. The presence of a germline BRCA mutation different from the one already detected at the tumor is not probable either, since the allelic fraction should be the same or higher. In addition, under a universal diagnostic strategy, in patients without a tumor BRCA1/2 mutation but with clinical suspicion of hereditary cancer, a germline testing was established.

Then, although an argument could be made for a higher prevalence of germline BRCA1/2 mutations in the cohort of NMHGOC patients, we consider that missing germline mutations due to the previous concerns would have been anecdotic, and that the estimated somatic and germline prevalence reliably reflect the real prevalence. Disparities between the real and estimated somatic/germline BRCA1/2 mutations prevalence are not expected in the cohort of prostate adenocarcinoma patients either, since the technology is capable of detecting CNV at the tumor. Concordance with the scientific literature supports these conclusions.

A meta-analysis of 14 studies has shown a more favorable prognosis for women with ovarian cancer associated with germline BRCA1/2 mutations, due to a greater sensitivity to platinum-based chemotherapy [13]. Germline or somatic BRCA1/2 mutations have also been associated to PARP (poly ADP-ribose polymerase) inhibitor activity [14-22]. Nowadays, it is a recommendation that all patients with non-mucinous epithelial ovarian cancer undergo germline BRCA1 and BRCA2 mutational analysis in the first instance for diagnostic, predictive and prognostic purposes; in patients who test negative for germline mutation, the analysis should be completed with somatic testing of tumor tissue [23]. This has been proven feasible with a streamlined oncologist-led BRCA germline testing and counseling, in order to save genetic counseling visits due to a shortage of genetic counselors; however, this model is difficult to reconcile with Spanish law which establishes that genetic counseling has to be performed by qualified professionals at accredited centers [5, 24].

Our series resembles the published data, demonstrating that tumor analyses, with the added possibility of revealing somatic mutations, detect more BRCA1/2 mutations than germline tests [7-11]. These findings endorse a reverse strategy. Starting the BRCA1/2 test at the tumor should detect more mutations in the first approach than the

germline analysis, improving prognostic and predictive yields. It could also serve as a molecular tumor screening for hereditary cancer diagnosis, selecting those tumors with BRCA mutations for testing the same gene alteration at the germline setting. This would lead to a more cost-effective diagnosis of HBOC, preserving accuracy while saving a lot of mandatory, legally imposed pre-counseling visits in our country, while also allowing a real streamlined process initiated by other specialists (since tumor testing has less ethical and legal implications), to be completed by genetic counselors when a germline analysis is required [5]. For the joint objective of HBOC diagnosis and treatment prediction, the reverse strategy spares and eases genetic counseling proceedings and genetic tests (Figure 2).

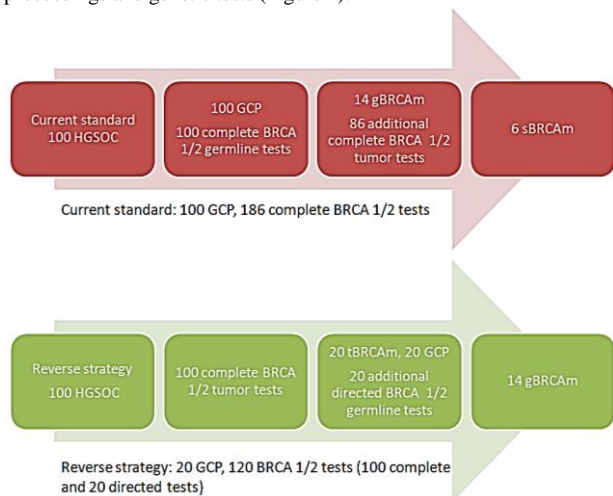


Figure 2: Advantages of the reverse strategy over the current standard. According to the published prevalence of germline and somatic BRCA mutations in high grade serous ovarian carcinoma (HGSOC), for hereditary breast and ovarian cancer (HBOC) diagnosis and predictive objectives, the reverse strategy would save 80 genetic counseling processes (GCP) and 66 genomic tests (simplifying 20 directed germline tests) in every 100 patients. For the same germline and somatic BRCA mutation diagnostic yield, the reverse strategy would be more cost-effective, with an easier and more rational sequence of steps in the clinical practice.

gBRCAm: germline BRCA mutation; sBRCAm: somatic BRCA mutation; tBRCAm: tumor BRCA mutation.

To reach full clinical utility validation and feasibility for HBOC diagnosis in our country (with stringent legal issues in the field), results should be confirmed in a wider, multicenter, prospective clinical trial of Spanish NMHGOC cases unselected for family history, employing technology capable of detecting BRCA CNV at the tumor setting and with complete germline BRCA testing in all the patients, to measure the real, possible loss of HBOC diagnoses.

Furthermore, it should be advisable to validate other reverse strategies, based on analyzing the genomic instability phenotype (somatic molecular hallmark of HRRD), given that three independent DNA-based measures of genomic instability have shown a predictive value for PARP-inhibitor treatment: loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST) [18-20, 25, 26]. HRDetect, a low coverage affordable whole genome sequencing (WGS) test that utilizes a multiple mutational signature to capture an

HRRD phenotype, has been developed in cohorts of ovarian, breast and pancreatic cancer samples as an accurate somatic molecular screening for HBOC diagnosis [27]. HRDetect also merits better validation in a reverse strategy for HBOC identification.

Our results in prostate adenocarcinoma point to the same diagnostic possibilities for HBOC. Considering the overall 17.4% prevalence of mutations in HR genes in 17,566 tumors with different histologies, and with the advent of an agnostic precision oncology based on multiplex gene panels that incorporate hereditary cancer genes and molecular profiles (HRRD, microsatellite instability, tumor mutational burden), a reverse strategy for hereditary cancer diagnosis is going to be imposed in some patients [28]. In this new scenario, genomic and molecular portrait results are a challenge for the inference of a possible germline mutation. In ovarian cancer, tumor BRCA pathogenic variants are usually driver, actionable mutations and must be discarded as hereditary (even with a low allelic fraction, unless there is good tumor purity and quantity); however, in other histologies with tumor HR gene mutations, it is advisable to demonstrate an HRRD for therapeutic objectives [29].

In conclusion, our study depicts the estimated somatic and germline BRCA1/2 mutation prevalence in two cohorts of non-mucinous high grade ovarian/fallopian tube/peritoneal extraovarian cancer patients and prostate adenocarcinoma patients. In agreement with published data, the tumor mutation rate is higher than germline mutation prevalence, raising the hypothesis of a more cost-effective analysis for predictive and diagnostic goals, with neoplastic tissue study as the first attempt.

Competing Interests

None.

Acknowledgments

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REFERENCES

1. Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C et al. (2012) BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol* 30: 2654-2663. [[Crossref](#)]
2. Arts-de Jong M, de Bock GH, van Asperen CJ, Mourits MJ, de Hullu JA (2016) Germline BRCA1/2 mutation testing is indicated in every patient with epithelial ovarian cancer: A systematic review. *Eur J Cancer* 61: 137-145. [[Crossref](#)]
3. Pritchard CC, Mateo J, Walsh MF, De Sarkar N, Abida W et al. (2016) Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N Engl J Med* 375: 443-453. [[Crossref](#)]

4. Castro E, Romero-Laorden N, del Pozo A, Lozano R, Medina A et al. (2019) PROREPAIR-B: A Prospective Cohort Study of the Impact of Germline DNA Repair Mutations on the Outcomes of Patients With Metastatic Castration-Resistant Prostate Cancer. *J Clin Oncol*; 37(6): 490-503. [[Crossref](#)]
5. Ley 14/2007, de 3 de Julio, de Investigación biomédica. BOE número 159, páginas 28826-28848. Documento BOE-A-2007-12945. [[Crossref](#)]
6. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H et al. (2015) DNA-repair defects and Olaparib in metastatic prostate cancer. *N Engl J Med* 373: 1697-1708. [[Crossref](#)]
7. Mandelker D, Zhang L, Kemel Y, Stadler ZK, Joseph V et al. (2017) Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA* 318: 825-835. [[Crossref](#)]
8. Bell D, Berchuck A, Birrer M, Chien J, Cramer D et al. (2011) Integrated genomic analyses of ovarian carcinoma. *Nature* 474: 609-615. [[Crossref](#)]
9. Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC et al. (2014) Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. *Clin Cancer Res* 20: 764-775. [[Crossref](#)]
10. Moschetta M, George A, Kaye SB, Banerjee S (2016) BRCA somatic mutations and epigenetic modifications in serous ovarian cancer. *Ann Oncol* 27: 1449-1455. [[Crossref](#)]
11. Dougherty BA, Lai Z, Hodgson DR, Orr MCM, Hawryluk M et al. (2017) Biological and clinical evidence for somatic mutations in BRCA1 and BRCA2 as predictive markers for olaparib response in high-grade serous ovarian cancers in the maintenance setting. *Oncotarget* 8: 43653-43661. [[Crossref](#)]
12. Judkins T, Rosenthal E, Arnell C, Burbidge LA, Geary W et al. (2012) Clinical significance of large rearrangements in BRCA1 and BRCA2. *Cancer* 118: 5210-5216. [[Crossref](#)]
13. Zhong Q, Peng HL, Zhao X, Zhang L, Hwang WT (2015) Effects of BRCA- and BRCA2-related mutations on ovarian and breast cancer survival: a meta-analysis. *Clin Cancer Res* 21: 211-220. [[Crossref](#)]
14. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I et al. (2014) Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomized phase 2 trial. *Lancet Oncol* 15: 852-861. [[Crossref](#)]
15. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I et al. (2012) Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med* 366: 1382-1392. [[Crossref](#)]
16. Ledermann JA, Harter P, Gourley C, Friedlander M, Vergote I et al. (2016) Overall survival in patients with platinum-sensitive recurrent serous ovarian cancer receiving olaparib maintenance monotherapy: an updated analysis from a randomized, placebo-controlled, double-blind, phase 2 trial. *Lancet Oncol* 17: 1579-1589. [[Crossref](#)]
17. Pujade-Lauraine E, Ledermann JA, Selle F, GebSKI V, Penson RT et al. (2017) Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomized, placebo-controlled, phase 3 trial. *Lancet Oncol* 18: 1274-1284. [[Crossref](#)]
18. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S et al. (2016) Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N Engl J Med* 375: 2154-2164. [[Crossref](#)]
19. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H et al. (2017) Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part1): an international, multicenter, open-label, phase 2 trial. *Lancet Oncol* 18: 75-87. [[Crossref](#)]
20. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A et al. (2017) Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 390: 1949-1961. [[Crossref](#)]
21. Moore K, Colombo N, Scambia G, Kim B-G, Oaknin A et al. (2018) Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med* 379: 2495-2505. [[Crossref](#)]
22. Robson M, Im SA, Senkus E, Xu B, Domchek SM et al. (2017) Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med* 377: 523-533. [[Crossref](#)]
23. Oaknin A, Guarch R, Barretina P, Hardisson D, González-Martín A (2018) Recommendations for biomarker testing in epithelial ovarian cancer: a National Consensus Statement by the Spanish Society of Pathology and the Spanish Society of Medical Oncology. *Clin Transl Oncol* 20: 274-285. [[Crossref](#)]
24. Colombo N, Huang G, Scambia G, Chalas E, Pignata S (2018) Evaluation of a Streamlined Oncologist-Led BRCA Mutation Testing and Counseling Model for Patients With Ovarian Cancer. *J Clin Oncol* 36: 1300-1307. [[Crossref](#)]
25. Timms KM, Abkevich V, Hughes E, Neff C, Reid J, Morris B et al. (2014) Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Res* 16: 475. [[Crossref](#)]
26. Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, et al. (2016) Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. *Clin Cancer Res* 22: 3764-3773. [[Crossref](#)]
27. Davies H, Glodzik D, Morganella S, Yates LR, Staaf J et al. (2017) HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat Med* 23: 517-525. [[Crossref](#)]
28. Heeke AL, Pishvaian MJ, Lynce F, Xiu J, Brody JR et al. (2018) Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types. *JCO Precision Oncology* 2: 1-13. [[Crossref](#)]
29. Agarwal N, Sokol ES, Lara P, Ross JS, Miller VA et al. (2018) Pan-cancer assessment of BRCA1/2 genomic alterations (GAs) by comprehensive genomic profiling (CGP) of tissue and circulating tumor DNA (ctDNA). *Annals of Oncology* 29.