Short communication

Multigene germline testing usefulness instead of BRCA1/2 single screening in triple negative breast cancer cases

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ABSTRACT

Triple negative breast cancer is considered as the worst aggressive subtype with poor prognosis. Recent studies suggest a hereditary component is involved in TNBC development, especially in young patients. However, genetic spectrum remains unclear. Our purpose was to evaluate the usefulness of multigene panel testing in triple negative patients compared to overall breast cancer cases as well as contributing to elucidate which genes are most implicated in triple negative subtype development. Two breast cancer cohorts, comprising 100 triple negative breast cancer patients and 100 patients with other breast cancer subtypes, were analyzed by Next-Generation Sequencing using an On-Demand panel which included 35 predisposition cancer genes associated with inherited cancer susceptibility. The percentage of germline pathogenic variant carriers was higher in the triple negative cohort. *ATM*, *PALB2*, *BRIP1* and *TP53* were the most non-BRCA mutated genes. Moreover, triple negative breast cancer patients without family history related who were identified as carriers were diagnosed at significantly earlier age. As conclusion, our study reinforces the usefulness of multigene panel testing in breast cancer cases but specifically in those with triple negative subtype regardless family history.

1. Introduction

Accounting for 15–20% of breast cancer (BC) cases diagnosed, triple negative breast cancer (TNBC), defined by the absence of estrogen and progesterone receptors expression and a lack of epidermal growth factor 2 receptor overexpression [1], is considered the most aggressive BC subtype [2]. Higher grade tumor with great molecular and immunological heterogeneity are among its main features [3]. TNBC patients are often younger or pre-menopausal but they are frequently diagnosed at a later stage of disease, contributing to get worse prognosis and 5 year-survival rates respected other BC subtypes [2]. An association between germline mutations and TNBC development has been reflected by several studies, especially in young patients [4]. Clinical guidelines from different countries, such as the National Comprehensive Cancer Network (NCCN) in the USA and Spain's Society of Medical Oncology (SEOM), simply recommend *BRCA1/2* testing for TNBC patients diagnosed at 60 years of age (NCCN) [5], 50 years of age (SEOM) [6], or younger. However, germline PVs in other genes have also recently been associated with TNBC [3,7].

2. Materials and methods

2.1. Patient samples

Two hundred individuals diagnosed with BC were enrolled in this study. This group comprised 100 TNBC cases and 100 BC samples without the TNBC phenotype, which we termed *non-TNBC*, selected by the regional hereditary cancer prevention program of Castilla y León (Spain). Ethical committee approval and informed consent were given that subclasses have been made based on the age of diagnosis and the associated family history.

2.2. DNA isolation

Genomic DNA from peripheral blood samples was automatically extracted using the MagnaPure Compact system (Roche) and "MagNa

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Abbreviations: BC, breast cancer; TNBC, triple negative breast cancer; ACO, age of cancer onset; FH, family history; PV, pathogenic variant.
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pure Compact Nucleid Acid Isolation Kit I" (Roche), following the manufacturer's instructions.

2.3. Predisposition cancer gene analysis by next-generation sequencing

Genomic DNA samples were sequenced by Ion Torrent technology (Thermo Fisher Scientific, Waltham, MA, USA) using an On-Demand panel (Thermo Fisher Scientific, Waltham, MA, USA), previously designed to screen small variants as single nucleotide variants (SNVs) and insertion/deletion variants (Indels) in other hereditary cancer syndromes [8]. It included 35 genes associated with inherited cancer susceptibility: *BRCA1, BRCA2, MLH1, MSH2, PMS2, EPCAM, MSH6, APC, KRAS, PTEN, BMPR1A, CDH1, SMAD4, CDK4, STK11, TP53, MUTYH, ATM, PALB2, ATR, FANCM, BLM, MRE11A, BARD1, PRKAR1A, BRIP1, FAM175A, CHEK2, RAD51C, RAD51D, RAD50, NBN, POLD1, MEN1* and *POLE*. Genomic DNA samples were checked for concentration using a Qubit 3.0 fluorometer (Thermo Fisher Scientific. The library and template preparation were performed using the automated Ion Chef™ instrument (Thermo Fisher Scientific) with the "Ion Ampliseq™ kit Chef DL8″ and "Ion Chip 520™ (Thermo Fisher Scientific) from 10 ng per genomic DNA sample, according to the manufacturer's instructions. The Ion Chip 520 obtained from template reaction was sequenced in Ion S5™ (Thermo Fisher Scientific) according to the manufacturer's instructions. The average value of total aligned reads was 7,398,788 (99, 9%). Sequencing results were aligned to the hg19 human reference genome and analyzed using the Ion Reporter Software Version 5.10 (Thermo Fisher Scientific) being filtered and selected those variants with 30x minimum coverage.

2.4. Variant classification

Variant classification was performed by searching on GnomAD (https://gnomad.broadinstitute.org) population database and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) variant database. We selected those were cataloged as pathogenic (class 5) or likely pathogenic (class 4) which were collectively termed as "pathogenic variant" (PV).

2.5. Statistical analysis

Statistical analysis was performed by SPSS software. The data were used to carried out descriptive statistics, including means, frequencies and percentages. The prevalence and distribution of the PV were analyzed among the fully tested individuals and specifically in cohorts by subtype. Chi-square test was performed to evaluate relationships between the categorical variables.

3. Results

3.1. Study population parameters

Cohorts' distribution is summarized in supplemental table 1. Despite our BC patients sample ranged from 25 to 50 age of cancer onset (ACO) the distribution was unequal, being majority the 40–50 ACO (71,42%) (Fig. 1A). Mean ACO was similar both TNBC and non-TNBC cohorts (43.51 years old vs. 41.26 years old). Further differences were found when subclassifying based on their family history (FH) associated (Fig. 1B). More than a half of the TNBC women (68%) had no FH related whereas HBOC FH related was the most prevalent subclass among non-TNBC patients.

3.2. Germline PV prevalence

Fourteen percent of BC patients we tested (28/200) turned out to be PV carriers. Focusing on TNBC stratification, percentage of PV carriers in those BC patients which exhibited the triple negative phenotype was 17% whereas only 11% of non-TNBC analyzed were PV carriers. Identified PV are described in supplemental table 2. Within the PV carriers set, BRCA1/2 prevalence was lower than other susceptibility genes analyzed (39.29% vs. 60.71%) which included *ATM, BRIP1, TP53, PALB2, RAD51D, MUTYH, APC* and *BLM* genes (Fig. 2). Subtype stratification revealed a higher frequency of BRCA1/2 PVs in the TNBC cohort than in the non-TNBC cohort (52.94% vs. 18.18%, respectively). However, they were not equally represented, supporting the existence of some correlation among TNBC subtype development and being a *BRCA1* PV carrier (p = 0.01), which was not found for *BRCA2* (p = 0.65). The prevalence of non-BRCA predisposition genes remained comparable
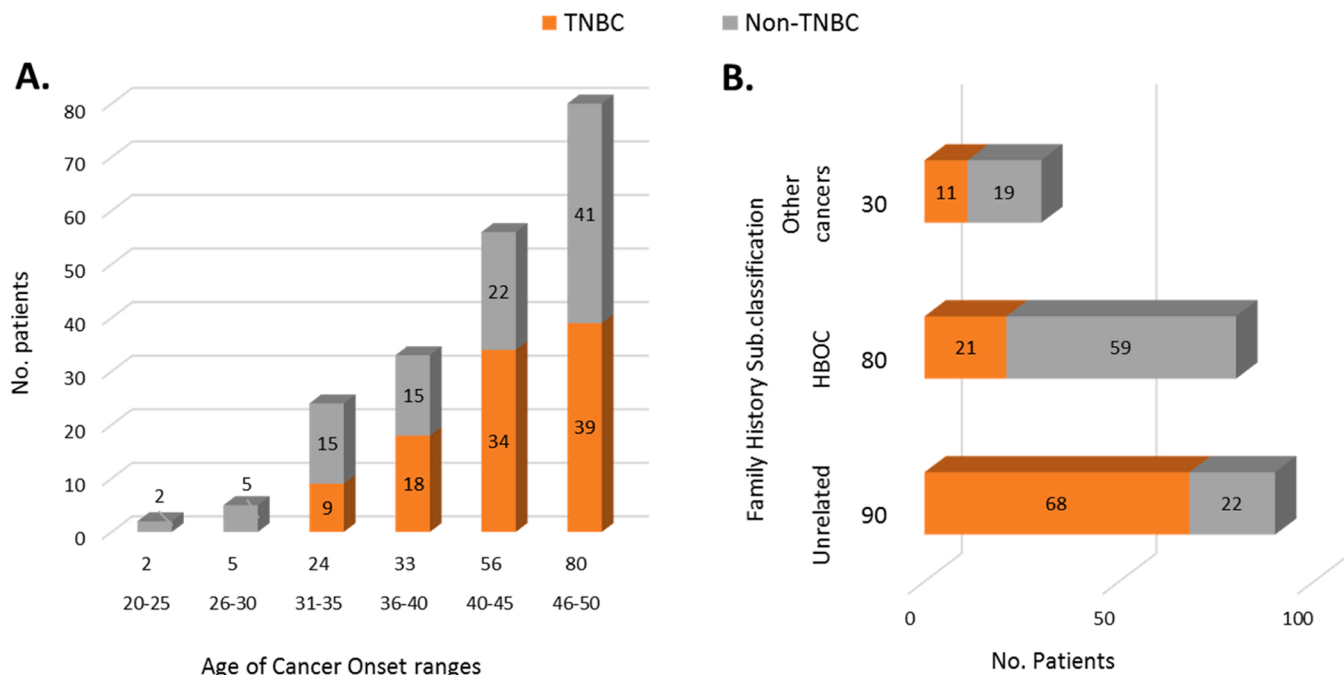

Fig. 1. Individuals' distribution according to age of cancer onset (A) and family history (B). HBOC Hereditary Breast and Ovarian Cancer.
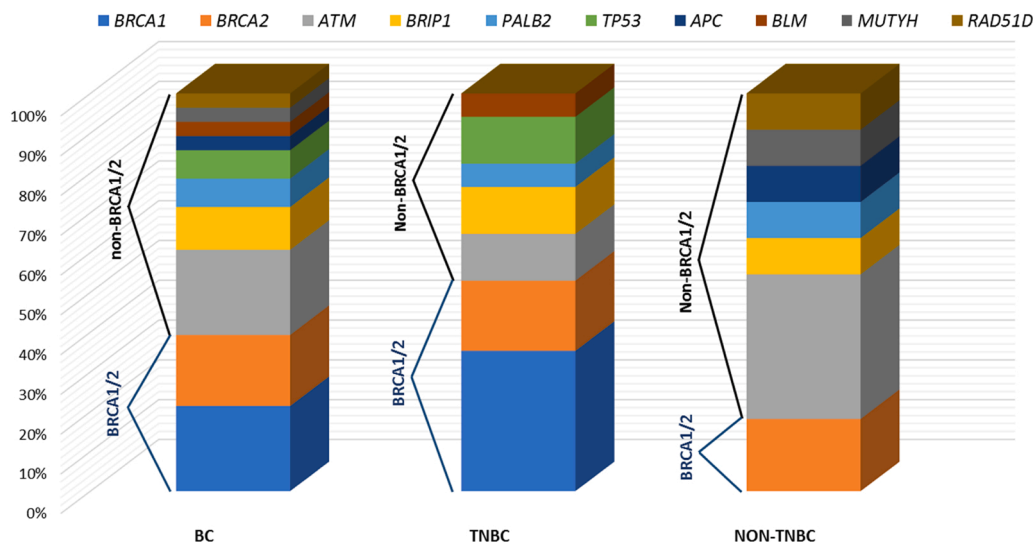
Fig. 2. Distribution of pathogenic variants for all BC women analyzed (left bar), and by TNBC/non-TNBC subtype. BC Breast cancer; TNBC Triple negative breast cancer.

between cohorts (p = 0.8), being *ATM* the most frequently mutated gene in this group, followed by *BRIP1*, *PALB2* and *TP53*.

3.3. PV carrier distribution according to age of cancer onset and family history associated

PV carrier frequencies remained comparable across ACO ranges despite the unequal distribution of our sample (Fig. 3A). Focusing on the cohorts, higher frequencies were detected in TNBC patients regardless of ACO (Fig. 3B).

In spìte of the uneven distribution, similar PV carrier frequencies were found in the FH-based subgroups (Fig. 3C-D). According to the statistical analysis, a positive FH associated (including both HBOC and other cancer FH) was significantly associated with carrying some PV in the TNBC cohort (p = 0.04). When both variables (ACO and FH) were combined, a significant association between early age of diagnosis and being a carrier of PV in TNBC without FH was found (p = 0.04) (Fig. 3E).

4. Discussion

Consistent with similar studies, our results support the fact that genes other than *BRCA1/2* are involved in BC development [4,7,9–11]. Therefore, the usefulness of panel gene testing over single *BRCA1/2* analysis when hereditary BC is suspected has been proved. Although the TNBC subtype showed a significant enriched prevalence of *BRCA1/2* PVs compared to the non-TNBC subtype (p = 0.03 *BRCA1/2*; p = 0.013 *BRCA1*), 45.05% of PVs were identified in genes other than BRCA. Therefore, multigene panel testing should be performed regardless of TN/non-TN subtype. Concerning ACO, our findings are consistent with the fact that *BRCA1* carriers usually had an earlier cancer onset than those *BRCA2* and non-BRCA carriers [10]. Moreover, TNBC patients without FH who were PV carriers had a significantly earlier ACO (Fig. 3E). As a consequence, our results justify the use of germline testing in TNBC cases, regardless of FH [12]. The addition of moderate susceptibility genes in hereditary BC screening routine may increase the ability to identify a large number of carriers who will benefit from specific treatments based on their mutated gene, and it could also guide surgical decisions [13]. Furthermore, this would improve cascade testing to their healthy relatives, who could be included in screening programs and they could even take decisions concerning prophylactic and reproductive measures [14].

In the present study, not all assessed genes were equally informative. PVs were detected in just 10 of the analyzed genes (Fig. 2, left bar). The gene spectrum identified differed moderately between subtypes. PVs in *BLM*, *BRCA1* and *TP53* genes were detected in TNBC patients (Fig. 2, middle and right bar) while *APC*, *MUTYH* and *RAD51D* PVs were only identified in the non-TNBC cohort. PVs from *ATM*, *BRCA2*, *BRIP1* and *PALB2* genes were found in both cohorts. Our research supports some correlation between *ATM* [9], *PALB2* [4,7,11], *BRIP1* [11,13] and *TP53* [11] with TNBC development but in contrast to similar studies [4], our results cannot point out any *BARD1* association.

Some weaknesses of multigene panel testing should be mentioned. First, the unclear genetic spectrum involved in the BC onset leads to discover incidental findings [15] and a greater identification of VUS by their use [16]. Likewise, although risk reduction measures are well established for healthy BRCA1/2 carriers, this is not the case for most of the moderate penetrance genes [17], delaying their management and triggering anxiety in some cases, especially when faced with VUS or incidental findings [18]. Further studies around the prevalence of *non-BRCA* susceptibility BC genes should be carried out to delineate their risk and involvement in BC onset. Moreover, the increased use of multigene germline screening may allow us to catalogue the high number of VUS identified in predisposition genes as not characterized as *BRCA1/2*. Finally, certain points of our study should be noted. Our results could be distorted due to the late ACO for most of the available samples (Fig. 1A). Another limitation was the lack of tumor characteristics and additional clinical information which could have been relevant in some cases, but unfortunately was unavailable. Moreover, the multigene panel we used was only capable of detecting small variants but not suitable for large genomic rearrangements such as copy number variants, which can also be involved in BC development.

In conclusion, deployment of multigene panel testing rather than solely *BRCA1/2* testing for BC women, and particularly at TNBC, is required. Furthermore, our results show that it ought to be independent of FH. However, extra susceptibility gene studies are required to find out the optimal genetic contribution, as this would reduce incidental findings and VUS occurrence. This work has contributed by identifying *ATM*, *PALB2*, *BRIP1*, and *TP53* in addition to *BRCA1/2*, as the most prevalent mutated genes in TNBC women.
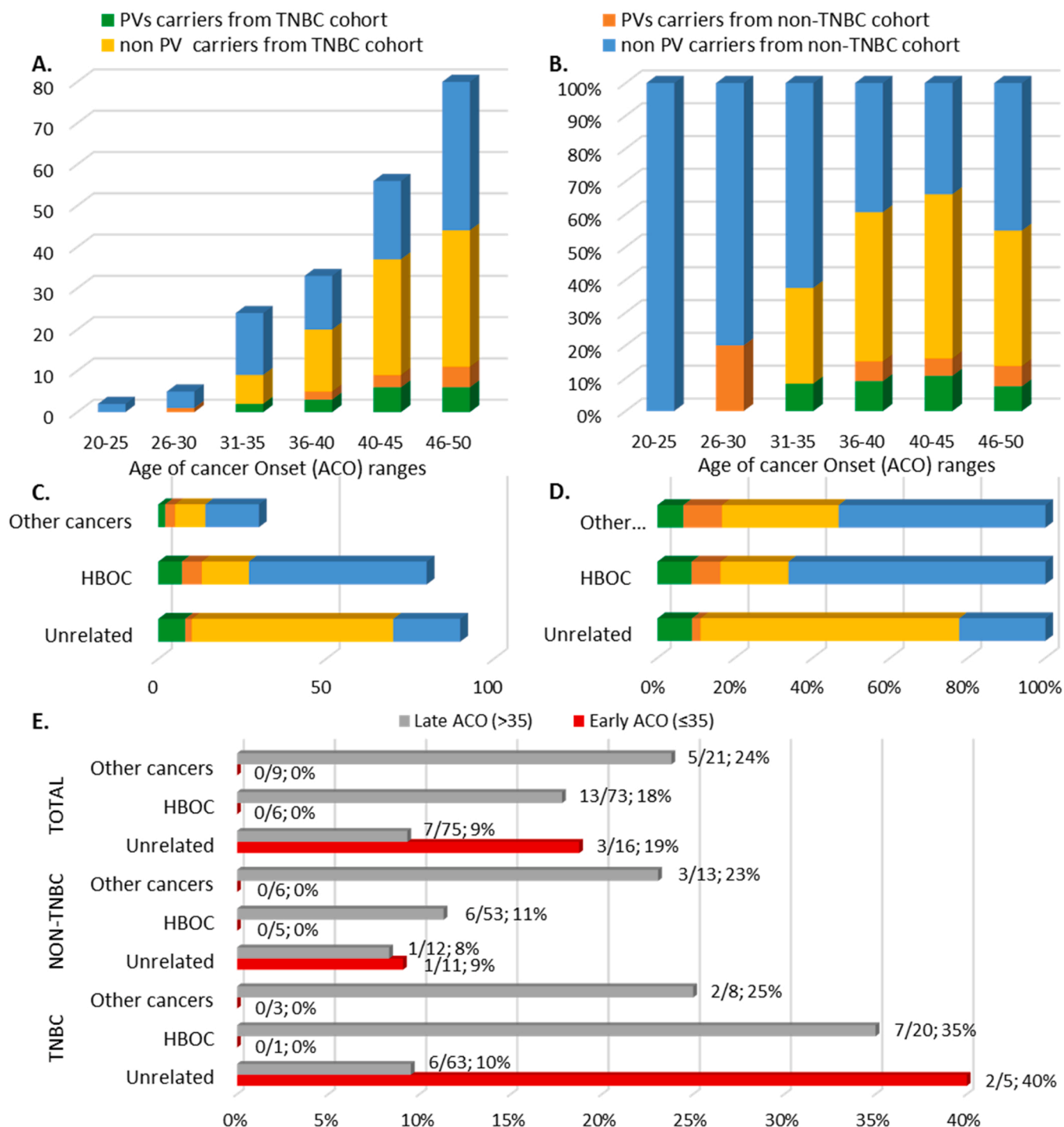
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Fig. 3. Distribution (A, C) and frequencies (B, D, E) of PV carriers according to age of cancer onset (A-B), family history associated (C-D) and both variables combination (E). ACO age of cancer onset; HBOC Hereditary Breast and Ovarian Cancer; TNBC Triple Negative Breast Cancer.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prp.2023.154514.

References

[1] P. Kumar, R. Aggarwal, An overview of triple-negative breast cancer, Arch. Gynecol. Obstet. 293 (2) (2016) 247–269.

[2] X. Li, J. Yang, L. Peng, A.A. Sahin, L. Huo, K.C. Ward, et al., Triple-negative breast cancer has worse overall survival and cause-specific survival than non-triple-negative breast cancer, Breast Cancer Res Treat. 161 (2) (2017) 279–287.

[3] J.L. da Silva, N.C. Cardoso Nunes, P. Izetti, G.G. de Mesquita, A.C. de Melo, Triple negative breast cancer: a thorough review of biomarkers. Critical Reviews in Oncology/Hematology, Elsevier Ireland Ltd, 2020. Vol. 145.

[4] H. Shimelis, H. LaDuca, C. Hu, S.N. Hart, J. Na, A. Thomas, et al., Triple-negative breast cancer risk genes identified by multigene hereditary cancer panel testing, J. Natl. Cancer Inst. 110 (8) (2018) 855–862.

[5] M.L. Telli, W.J. Gradishar, J.H. Ward, NCCN guidelines updates: breast cancer, J. Natl. Compr. Canc Netw. 17 (55) (2019) 552–555.

[6] S. González-Santiago, T. Ramón y Cajal, E. Aguirre, J.E. Alés-Martínez, R. Andrés, J. Balmaña, et al., SEOM clinical guidelines in hereditary breast and ovarian cancer (2019), Clin. Transl. Oncol. 22 (2) (2020) 193–200.

[7] F.J. Couch, S.N. Hart, P. Sharma, A.E. Toland, X. Wang, P. Miron, et al., Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer, J. Clin. Oncol. 33 (4) (2015) 304–311.

[8] C. Velázquez, E. Lastra, F. Avila Cobos, L. Abella, V. De La Cruz, B.A. Hernando, et al., A comprehensive custom panel evaluation for routine hereditary cancer testing: Improving the yield of germline mutation detection, J. Transl. Med [Internet] 18 (1) (2020) 1–12, https://doi.org/10.1186/s12967-020-02391-z.

[9] N. Tung, N.U. Lin, J. Kidd, B.A. Allen, N. Singh, R.J. Wenstrup, et al., Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer, J. Clin. Oncol. 34 (13) (2016) 1460–1468.

[10] Y. Chávarri-Guerra, C.A. Marcum, C.B. Hendricks, D. Wilbur, T. Cescon, C. Hake, et al., Breast cancer associated pathogenic variants among women 61 years and older with triple negative breast cancer, J. Geriatr. Oncol. 12 (5) (2021) 749–751.

[11] M. Zanti, M.A. Loizidou, K. Michailidou, P. Pirpa, C. Machattou, Y. Marcou, et al., Ngs panel testing of triple-negative breast cancer patients in Cyprus: A study of BRCA-negative cases, Cancers 12 (11) (2020) 1–11.

[12] J. Hoyer, G. Vasileiou, S. Uebe, M. Wunderle, C. Kraus, P.A. Fasching, et al., Addition of triple negativity of breast cancer as an indicator for germline mutations in predisposing genes increases sensitivity of clinical selection criteria, BMC Cancer 18 (1) (2018).

[13] R. Bernstein-Molho, E. Evron, R. Yerushalmi, S. Paluch-Shimon, Genetic testing in patients with triple-negative or hereditary breast cancer, Curr. Opin. Oncol. NLM Vol. 33 (2021) 584–590.

[14] S.A. Metcalfe, Genetic counselling, patient education, and informed decision-making in the genomic era, Semin Fetal Neonatal Med [Internet] 23 (2) (2018) 142–149, https://doi.org/10.1016/j.siny.2017.11.010.

[15] N. Maani, K. Panabaker, J.M. McCuaig, K. Buckley, K. Semotiuk, K.M. Farncombe, et al., Incidental findings from cancer next generation sequencing panels, npj Genom. Med [Internet] (1) (2021) 6, https://doi.org/10.1038/s41525-021-00224-6.

[16] G. Federici, S. Soddu, Variants of uncertain significance in the era of high-throughput genome sequencing: a lesson from breast and ovary cancers, J. Exp. Clin. Cancer Res 39 (1) (2020) 1–12.

[17] R. Graffeo, H.Q. Rana, F. Conforti, B. Bonanni, M.J. Cardoso, S. Paluch-Shimon, et al., Moderate penetrance genes complicate genetic testing for breast cancer diagnosis: ATM, CHEK2, BARD1 and RAD51D, Breast (2022).

[18] M. Wright, V. Menon, L. Taylor, M. Shashidharan, T. Westercamp, C.A. Ternent, Factors predicting reclassification of variants of unknown significance, Am. J. Surg. 216 (6) (2018) 1148–1154, https://doi.org/10.1016/j.amjsurg.2018.08.008.