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ORIGINAL ARTICLE

Breast, ovarian and other site cancer patients with BRCA 1/2 mutations: Data from Turkish multicenter retrospective study

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Summary

Purpose: *To demonstrate the clinical and demographic findings of the patients harboring BRCA1/2 mutations with breast, genital tract, prostate and pancreas cancers.*

Methods: *The results of sequencing analysis of 200 cancer patients (190 women, 10 men) who had been directed to genetic counseling with an indication BRCA1/ BRCA2 testing from different regions across 9 medical oncology centers were retrospectively analyzed.*

Results: *A total of 200 consecutive cancer patients who harbored BRCA1/BRCA2 mutation [130 (65%) patients harbored BRCA 1 mutation, and 70 harbored BRCA 2 mutation] were included. Of these, 64.0% had breast cancer, 31.5% had genital cancers, 3.5% had prostate and 1.0% had pancreatic cancers. The age at diagnosis [57 (IQR 50-66) years] of parents who had BRCA mutant cancer was higher than the age of their children who had BRCA mutant cancer [median age 45 (IQR 38-45) years]. BRCA2 carriers with ovarian cancer had favorable survival outcomes. In ovarian cancer patients, progression-free survival longer than 12 months was significantly more frequent in BRCA2 carriers compared with those in BRCA1 carriers.*

Conclusions: *Newly diagnosed BRCA 1/2 carriers with cancers were younger than their parents who harbored BRCA mutation with cancer. The findings from Turkish BRCA 1/2 associated cancer patients suggest that earlier onset of the screening program and genetic counseling of BRCA associated patients and their family members are essential to earlier disease diagnosis and to prevent disease occurrence as well.*

Key words: *BRCA1, BRCA2, breast, pancreas, genital cancers, prostate, pancreas*

Introduction

Every cell has DNA damage response mechanisms that protect the genome against the harmful effects of mutations. DNA double-strand breaks are a very dangerous form of DNA damage and can be repaired by homologous recombination repair which includes breast cancer susceptibility genes

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BRCA1 and BRCA2. These genes act as tumor suppressors to promote homologous recombination repair mechanism and their inherited mutations result in homologous recombination repair deficiency and leading to significant lifetime risks of breast, ovarian, and other cancers [1].

BRCA-related hereditary breast, ovarian and other cancers have inherited autosomal dominant condition, for which early identification and intervention have a meaningful potential for clinical actionability and a positive impact on public health. In routine practice, genetic testing for these conditions is based on family history and other demographic characteristics [2,3]. Genetic counseling should be given to patients with BRCA 1/2 carriers and other family members. Due to the fact that BRCA-related cancers are diagnosed at an earlier age than non-BRCA 1/2 carriers, earlier screening program protocols are recommended. On the other hand, there is not enough data on whether the diagnosis age of BRCA1/2 mutant patients is different than their parents who had BRCA1/2 carriers with cancer.

In this study, we aimed to demonstrate the clinical and demographic findings of the patients harboring BRCA1/2 mutations with breast, genital tract, prostate, and pancreas cancer in Turkish patients.

Methods

Study subjects

This retrospective multicenter study includes the results of sequencing analysis of 200 patients (190 women, 10 men) who have been directed to genetic counseling with an indication *BRCA1/ BRCA2* testing from different regions of Turkey. This study was approved by the local ethics committee.

DNA isolation

Genomic DNA was isolated from peripheral blood samples by using the EasyOne DNA isolation system (Qiagen, Hilden, Germany) and isolated DNA samples were assessed spectrophotometrically with NanoDrop (Thermo Fisher Scientific). Samples with A260/280 values between 1.8-2.0 were used for Next-Generation Sequencing. Low quality DNA samples were re-extracted from stored blood samples.

Next generation sequencing (NGS)

For NGS, QIAseq Targeted Amplicon Panel (Qiagen, Hilden, Germany), covering the coding regions of *BRCA1* and *BRCA2* genes with 20bp intron padding primers was used. Amplicon libraries were prepared according to the instructions of the manufacturer (Qiagen, Hilden, Germany). Pooled libraries were sequenced on the MiSeq System (Illumina, San Diego, CA, USA) following the target enrichment process. Fastq generation was performed on MiseqReporter Software (Illumina, San Diego, CA). Quality control of sequenced amplicons and variant call format (vcf) file generation were performed using QCI analysis software (Qiagen, Hilden, Germany). Variant analysis has been performed using Ingenuity and Clinical Insight Softwares (Qiagen, Hilden, Germany), and all rare and novel variants were visually controlled by using IGV 2.4.8 (www.broadinstitute.com). Segregation analysis of family members were performed using Sanger Sequencing with in-house designed primer sets covering the mutation regions.

Data analysis in silico predictions and variant classification

The latest versions of gnomAD [4], dbSNP [5], and ClinVar [6] databases were considered for comparing known variant frequencies. HGMD [7] and literature accessions were also considered. ACMG 2015 [8] guidelines were used for final classification of the variants .

All statistical analyses were performed using IBM SPSS ver. 22 (SPSS Inc., Chicago, IL). Data were pre-

Table 1. Clinical and demographic findings of the study subjects

Findings	*n (%)*
Age, years	
Median (Interquartile range)	45 (38-54)
Gender	
Female	190 (95.0)
Male	10 (5.0)
ECOG-PS	
0	162 (81.0)
1	38 (19.0)
Primary tumor	
Breast	128 (64.0)
Genital	63 (31.5)
Prostate	7 (3.5)
Pancreas	2 (1.0)
Family history	91 (45.5)
Degree of relatives	
First-degree	67 (33.5)
Second degree	22 (11.0)
Third degree	2 (1.0)
Diagnosis age of relatives, median (IQR)	
Parent diagnosis	57 (50-66)
Sibling diagnosis	44.5 (35-49)
Second relatives	40 (35.5-48.5)
Multiple primary tumor	12 (6.0)
Synchronous	1 (0.5)
Metachronous	11 (5.5)
Multiple primary tumor site	
Breast-ovary	11 (5.5)
BRCA	
BRCA1	130 (65.0)
BRCA2	70 (35.0)

sented as median (25th-75th interquartile range). Categorical variables were reported as frequencies and group percentages. Progression-free survival was defined as the time from the date of initial diagnosis to disease progression or death due to any cause.

Results

Study patients

A total of 200 BRCA mutant patients were analyzed across 9 medical oncology centers. Table 1 shows the clinical and demographic characteristics of the study subjects. Of these, 130 (65%) patients harbored BRCA 1 mutation, and 70 harbored BRCA 2 mutation. The median age at diagnosis was 45 years (IQR: 38-54). About 45.5% of the patients had a family history. The presence of malignancy was 33.5% in first-degree relatives and 11.0% in second-degree relatives. Of these, parent BRCA1 or 2 mutation was 14% (n=28) and the diagnosis age of parent was higher than the diagnosis age of the study subjects (Figure 1). The diagnosis ages of sibling and second-degree relatives were 44.5 and 40 years, respectively. Only 1 patient had a synchronous disease and 11 patients had metachronous (breast and ovary) multiple primary disease.

Breast cancer

Table 2 shows demographic and clinical characteristics of breast cancer patients who harbored BRCA mutation. Breast cancer prevalence was 67% (95% CI 60.2 to 73.8 percent) in all patients and their median age was 41.5 years (34-50), and patients diagnosed with breast cancer under 45 years was much more in BRCA1 mutant than BRCA2 mutants (73.4% vs 55.1%, p=0.03, respectively). Luminal disease (without HER-2 positivity 44.5%, with HER-2 positivity 7.0%), triple-negative disease

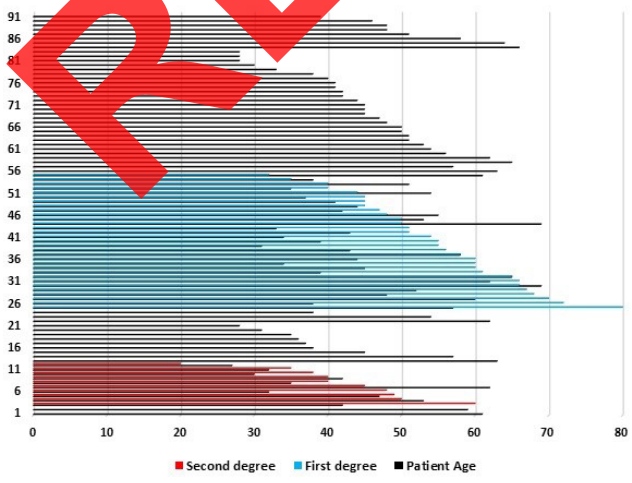

Figure 1. Diagnosis age of all patients and their relatives.

Table 2. Clinical and demographic data of breast cancer patients

Findings	*n (%)*
Age, years	
Median (Interquartile range)	41.5 (34-50)
Gender	
Female	126 (98.4)
Male	2 (1.6)
ECOG-PS	
0	114 (89.1)
1	14 (10.9)
Primary tumor size (T)	
0-2 cm –T1	79 (79.0)
2-5 cm- T2	15 (15.0)
5 cm and above-T3	4 (4.0)
Lymph node metastasis	61 (47.7)
Stage	
Stage I	30 (23.4)
Stage II	53 (41.4)
Stage III	29 (22.7)
Stage IV	14 (10.9)
Histopathology	
ER, %, median (IQR)	45 (0-90)
PgR, %, median (IQR)	0 (0-65)
CerbB2, IHC	
1+	17 (13.3)
2+	22 (17.2)
3+	8 (6.3)
cerbB2, FISH	12 (9.4)
ki-67, %, median (IQR)	30 (15-50)
Subtypes	
Triple negative	56 (43.8)
Luminal Her2-	57 (44.5)
Luminal Her2+	9 (7.0)
Her2+	3 (2.3)
Tumor location	
Right	65 (50.8)
Left	58 (45.3)
Bilateral	5 (3.9)
De novo metastasis	14 (10.9)
Metastasis site	
Lung	10 (7.8)
Bone	18 (14.1)
Liver	8 (6.3)
Lymph node	4 (3.1)
Family history	64 (50.0)
Diagnosis age of relatives, median (IQR)	
Parent diagnosis (n=15)	51 (46-57.5)
Sibling diagnosis (n=7)	44 (35-49)

(43.8%), and only HER-2 mutant (2.3%) disease were common subtypes. TNBC was the most common (60.5%) histopathology of BRCA1 mutant patients and hormone receptor-positive disease was the most common (79.6%) type of BRCA2 mutant patients (p<0.001). About 10.9% of the patients were diagnosed at the metastatic stage, without difference between BRCA1 vs BRCA2 mutant patients. Half of the patients had positive family history regarding breast and ovarian cancer. Parent diagnosis age of BRCA related cancer was 51 years (46-57.5), while it was 44 years (35-49) in sibling who had BRCA related cancer (Figure 2).

Table 3. Clinical characersitics of the genital site tumors

Characersitics	*n (%)*
Age, years	
Median (Interquartile range)	50 (44-59)
ECOG-PS	
0	43 (68.3)
1	20 (31.7)
Tumor Location	
Ovary	58 (92.1)
Endometrium	2 (3.2)
Peritoneum	1 (1.6)
FIGO stage	
Stage I	12 (19.0)
Stage II	9 (14.3)
Stage III	26 (41.3)
Stage IV	12 (19.0)
De novo metastasis	20 (31.7)
Histopathology	
Serous	48 (76.2)
Endometrioid	9 (14.3)
Serous+Endometrioid	4 (6.3)
Postop residual disease	13 (20.6)
Ca125 at diagnosis	
Median (Interquartile range)	155 (41-560)
BRCA	
BRCA1	49 (77.8)
BRCA2	14 (22.2)
Platinum-based therapy cycles	
Median (Interquartile range)	6 (6-6)
Platinum-therapy response, n (%)	
CR	43 (68.3)
PR	12 (19.2)
PFS of platinum based regimen (first-line)	
<6 months	0
6-12 months	14 (22.2)
>12 months	28 (44.4)
Platinum based line number	
Median (minimum-maximum)	1 (1-6)
Family history	24 (38.1)
Diagnosis age of relatives, median (IQR)	
Parent diagnosis	63 (58-68)

Genital cancer

Table 3 shows the clinical and demographic characteristics of BRCA patients who had genital site tumors. The median age was 50 years (44-59). Ovarian cancer was the most common (92.1%) primary site, endometrium (3.2%), and peritoneum (1.6%) were detected as well. Serous adenocarcinoma was the most common histopathology and 14.3% of the patients had endometrioid adenocarcinoma. About 77.8% of them had BRCA1 mutation and 22.2% had BRCA2 mutation. Progression-free survival longer than 12 months was significantly more frequent in BRCA2 (100%) carriers compared with those in BRCA1 (56.3%) carriers (p=0.01). About 38.1% of them had a positive family history. Parent diagnosis age of BRCA related cancer was 63 years (58-68; Figure 3).

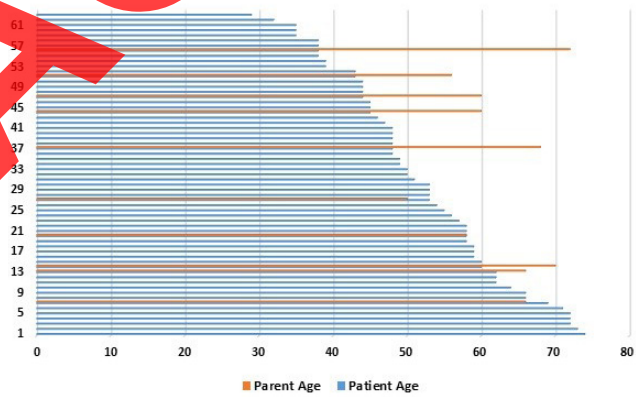

Figure 2. Diagnosis age of breast cancer patients and their relatives.

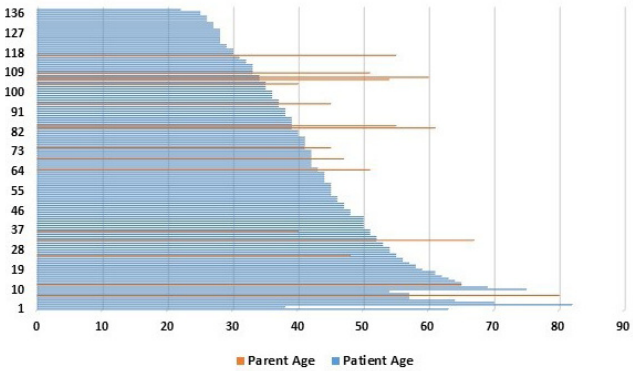

Figure 3. Diagnosis age of ovarian cancer patients and their relatives.

Table 4. Clinical and demographic findings of patients with prostate cancer

Findings	*n (%)*
Age, years	
Median (Interquartile range)	57 (57-60)
De novo metastasis	3 (42.9)
PSA	47 (14-74)
mCRPC	7 (100)
Time from metastasis to CRPC status (months)	28 (14-58)
Treatment line settings	
Docetaxel at 1 line	7 (100)
Enzalutamid at 2 line	5 (71.4)
Abiraterone at 2 line	1 (14.2)
Lutesyum at 3 line	3 (42.9)
Olaparib at 3 line	5 (71.4)
Docetaxel PFS (months)	13 (12-14)
Postdocetaxel treatment options	
Enzalutamide	5 (71.4)
Abiraterone	1 (14.2)
Cabazitaxel	1 (14.2)

Other

Table 4 shows data about the BRCA related prostate cancer patients. The median age was 57 years (57-60). All of the patients were diagnosed at the castration-resistant time. The median time from metastasis to castration-resistant status was 28 months (14-58). On the other hand, only 2 male patients had BRCA related pancreas cancer. The primary tumor was located at the corpus of the pancreas.

Discussion

This multicenter study, in which we assessed clinical and demographic characteristics of 200 patients who harbored BRCA1 or 2 mutation, demonstrated comparable findings with the literature. In addition, the diagnosis age of patients who harbored BRCA1/2 mutation was younger than the diagnosed age of their parents who harboring BRCA1/2 mutation with cancer. We suggest that the family members of the patients who harbored BRCA mutation should be alerted to be aware of this issue and genetic counseling should be given earlier.

Breast cancer is the most frequently diagnosed cancer in women. Although most of the newly diagnosed cases are sporadic, germline variants account for a small percentage of breast cancer [9]. Breast cancer type 1 or 2 mutations (BRCA1 and BRCA2) constitute the majority of hereditary ovarian and breast cancer and their identified pathogenic alterations are characterized by an autosomal dominant pattern of highly penetrant germline inheritance. A prospective cohort study showed that cumulative breast cancer risk was 72% (95% CI 65 to 79) in BRCA1 mutation carriers and 69% (95% CI 61 to 77) in BRCA2 mutation carriers [10]. Early-onset breast cancer is more prominent in patients who had BRCA related breast cancer [11]. Additionally, breast cancer incidence was noted to rise in early adulthood until 30 to 40 years for *BRCA1* carriers and until 40 to 50 years for *BRCA2* carriers [10,12]. In our study, the median age at initial diagnosis was 41.5 years (34-50) and breast cancer patients under 45 years were significantly much more in BRCA1 mutant than BRCA2 mutant. Family history is a risk factor for breast cancer and its incidence varies between BRCA related cancer patients [9,13]. O'Shaughnessy et al showed that family history was present in 45.5% of BRCA mutant breast cancer patients [9]. In our study, family history was 50%. Moreover, we found that patients with BRCA mutant breast cancer were diagnosed at an earlier age compared to their BRCA mutant parent's diagnosis age. Breast cancer screening programs and prior knowing their hereditary risk factors from parents might be the reason for this difference. In addition, average-risk screening protocols for breast cancer screening such as mammography at age 50 in women, do not adequately detect disease early enough for BRCA mutant individuals [3,14]. Assessment of newly diagnosed breast cancer patients for hereditary cancer conditions and genetic counseling for high-risk patients should be kept in mind in every newly diagnosed patient. On the other hand, triple-negative breast cancer histopathology was more frequent in BRCA mutant patients, especially in BRCA1 mutant patients [15-17]. In addition, it was shown that hormone-receptor-positive disease is more frequently associated with BRCA2 mutant breast cancer [18]. Similarly, we showed that triple negative breast cancer was the most common histopathology of BRCA1 mutant patients and hormone receptor-positive disease was the most common type of BRCA2 mutant patients. Additionally, female breast cancer patients ≤45 years old were significantly more in the BRCA1 mutant group and the most common histopathology was triple-negative disease. Patients above 45 years old, triple-negative histology in BRCA1 mutant patients were comparable to those in BRCA2 breast cancer patients.

Female genital tract cancers and their relation with BRCA1/2 mutations are most frequently observed with ovarian cancers. Apart from epithelial ovarian cancer, peritoneum, fallopian tube, peritoneum and endometrium are also less frequently

affected. One study from the Japanese HBOC consortium showed that the fallopian tube and peritoneum as a primary tumor site was less than 10% of BRCA1 mutant patients and was significantly higher in BRCA2 compared with BRCA2 mutant patients [19]. In our study, 4.8 of BRCA1/2 mutant patients had primary endometrium and peritoneal cancer sites, and all of them were diagnosed in the BRCA1 mutant variant. Germline BRCA1/2 mutations related to epithelial ovarian cancer are consisted of at least 10% of newly diagnosed cases and its cumulative risk by 80 years of age was 44% for BRCA1 mutant carriers and 17% for BRCA2 mutant carriers [10]. The histopathology of BRCA 1/2 mutant ovarian cancer is mainly serous adenocarcinoma [20]. On the other hand, a European study from Lakhani et al showed that endometrioid histology was the second common histology of ovarian cancers in BRCA1 and BRCA2 carriers [21]. Similarly, we showed that serous carcinoma and endometrioid carcinoma histologies were the main histology types of BRCA1/2 carriers. BRCA1/2 mutation status affects both progression-free survival and overall survival [22]. Firstly, it was shown that ovarian cancers in BRCA1/2 mutant carriers had favorable survival outcomes compared with non-carrier [23-25]. Platinum sensitivity, repeatedly responded to platin-based regimens and longer duration of response might play important role in favorable survival advantage in BRCA1/2 carriers with ovarian cancer patients. With the emergence of new treatment options, such as poly (ADP-ribose) polymerase (PARP) inhibitors, it is thought that BRCA 1/2 carriers with ovarian cancer patients will benefit from these options. Additionally, BRCA2 carriers with ovarian cancer had favorable survival outcomes [22,26]. Similarly, we revealed that progression-free survival longer than 12 months was significantly more frequent in BRCA2 carriers compared with those in BRCA1 carriers. Age at diagnosis was also found to be an independent risk factor associated with survival [24]. It is not clear whether age at diagnosis in ovarian cancer patients harboring BRCA1/2 mutations differ from non-carriers. It was shown BRCA1 mutant ovarian cancer patients were younger compared with non-carriers, but it was not observed for BRCA2 carriers [23]. Another study showed that age at diagnosis in ovarian cancer patients harboring BRCA1/2 mutation was comparable to non-carriers [25]. In our study, we revealed that the age of diagnosis of ovarian cancer patients harboring BRCA mutations was younger than their parents' age of diagnosis of BRCA-associated cancer. Due to the fact that there is no evidence-based effective screening program for ovarian cancer, genetic counseling of all ovarian cancer patients diagnosed<70 years may help the early diagnosis of BRCA1/2 carriers and may enhance the prevention of disease occurrence.

The frequency of germline HRR deficiency-related mutations in metastatic prostate cancer was found to be around 12% according to one study, and BRCA2 was the most common of these mutations with 5.3%. BRCA1 mutation frequency was found to be 1% [27]. Prostate cancers with these mutations may have a worse prognosis and overall survival compared to those without such mutations, while with appropriate genomic targeted therapies (such as PARP inhibitors, platinum-based therapies) they may have better responses [28-30]. The median age of our patients was 57 and they were 10 years younger than the patients in Phase 1/2/3 studies [31-33] in which the efficacy of Olaparib in patients with BRCA mutation was evaluated. As expected, approximately half of our patients had metastatic disease at the time of diagnosis, consistent with the course of more aggressive disease in patients with BRCA mutation, and the time to progression to the CRPC period was short (approximately 28 months). Both the *de novo* metastatic disease rate and the time until CRPC were found to be consistent with the literature. If we examine 7 castration-resistant prostate cancer patients who constituted our cohort, all of these patients received docetaxel and interestingly, the use of docetaxel in these patients had much better results than docetaxel's own castration-resistant prostate cancer 1st line treatment phase 3 progression-free survival results (13 months vs. 9 months, respectively) [34]. We know that in cancers with BRCA mutations, very good treatment responses are obtained with platinum treatments. It is unknown whether there is such a treatment response situation between docetaxel and the BRCA mutation. This situation requires more detailed research. In our study, the disease was more aggressive in BRCA mutant patients (young age, high *de novo* metastasis rate). Therefore, in terms of prostate cancer screening in carriers with this mutation, especially BRCA2 mutation, the use of multiparametric MRI should also be considered, except for monitoring with PSA alone.

The incidence of pancreatic cancer is increasing in developed and developing countries. Some syndromes cause a genetic predisposition for this cancer. There is a higher level of evidence that BRCA2 is associated with an increased risk for this cancer than for BRCA1. In BRCA2 mutation carriers, the risk of pancreatic cancer is 3.5-10 times (1.87-6.58) increased [35,36]. No relationship could be demonstrated between pancreatic cancer and germline pathological variant (eg BRCA1/2) car-

riage in terms of age, family history, or disease stage. It was also not found that there was an independent relationship between overall survival in those with pathologic mutations. It has been shown that there is a favorable trend in overall survival with platinum-based therapies in patients with HRR, which appears to be a predictive factor for PARP inhibitor maintenance therapies.

There are several limitations in our study. First, retrospective clinical data of BRCA 1/2 mutant patients from medical records has disadvantages to control for all potential confounding bias. We guess these results suggest selection and institutional bias due to actively conducted genetic testing by medical genetics specialists at different medical centers. Despite these limitations, a noteworthy strength of our study is that the diagnosis age of patients who harbored BRCA1/2 mutation was younger than the diagnosed age of their parents harboring BRCA1/2 mutation with cancer and our study findings were consistent with the literature.

In conclusion, newly diagnosed BRCA 1/2 carriers with cancers were younger than their parents harboring BRCA mutation with cancer. The findings from Turkish BRCA 1/2 associated cancer patients suggest that earlier onset of the screening program and genetic counseling of BRCA associated patients and their family members are essential to diagnose earlier and to prevent disease occurrence as well.

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Conflict of interests

The authors declared no potential conflicts of interest for the research, authorship, and/or publication of this article.

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