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Delineating the relationship between circulating osteoprotegerin and bone health in women with a pathogenic variant in *BRCA1*: A cross-sectional analysis

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ABSTRACT

Purpose: Osteoprotegerin (OPG) plays an important role in the inhibition of osteoclast formation and bone resorption. Studies have reported lower OPG levels among women with a pathogenic variant (mutation) in the *BRCA1* gene, and thus, may be at greater risk for skeletal bone loss. Thus, we investigated the association between circulating OPG and two validated markers of bone health: 1) bone fracture risk score (FRAX) and 2) bone mineral density (BMD), among *BRCA* mutation carriers.

Methods: Women with a blood sample and clinical data were included in this analysis. An enzyme-linked immunosorbent assay (ELISA) was used to quantify serum OPG (pg/mL) and the 10-year risk of *major* osteoporotic fracture (FRAXmajor) and *hip* fracture (FRAXhip) (%) was estimated using a web-based algorithm. For a subset of women, lumbar spine BMD was previously assessed by dual x-ray absorptiometry (DXA)(T-score). A Mann–Whitney *U* test was used to evaluate the association between OPG and FRAX score, while linear regression was used to assess the association of OPG and BMD.

Results: Among 701 women with a *BRCA1* mutation, there was a significant (and unexpected) positive association between OPG levels and FRAX score (FRAXmajor: 2.12 (low OPG) vs. 2.53 (high OPG) $P < 0.0001$; FRAXhip: 0.27 (low OPG) vs. 0.44 (high OPG) $P < 0.0001$). In a subset with BMD measurement ($n = 50$), low serum OPG was associated with a significantly lower BMD T-score (-1.069 vs. -0.318; $P = 0.04$).

Conclusion: Our findings suggest that women with inherently lower OPG may be at risk of lower BMD, the gold standard marker of bone disease. Due to the young age of our cohort, on-going studies are warranted to re-evaluate the association between OPG and FRAX in *BRCA* mutation carriers.

1. Introduction

Women who inherit a pathogenic or likely pathogenic variant ('mutation' hereafter) in one of the two breast cancer susceptibility genes, *BRCA1* or *BRCA2*, face high lifetime risks of developing breast and ovarian cancer (Kuchenbaecker et al., 2017). Emerging evidence suggests that dysregulation of the receptor activator of nuclear factor κB (RANK)/RANK ligand (RANKL) – signaling pathway is implicated in *brca1*-associated mammary carcinogenesis (Widschwendter et al., 2015; Nolan et al., 2016; Odén et al., 2016). Specifically, Widschwendter and colleagues previously showed significantly lower circulating levels of osteoprotegerin (OPG) among women with a *BRCA* mutation compared to non-carrier controls. OPG is the endogenous decoy receptor for RANKL and, thus, antagonizes RANK-RANKL signaling (Nagy and

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Penninger, 2015). OPG is well-characterized to promote bone health through RANKL-inhibition by blocking the commitment of precurser cells to the osteoclast lineage, thereby preventing osteoclast maturation and reducing bone resorption (Simonet et al., 1997; Boyce and Xing, 2008; Boyce and Xing, 2007).

Among healthy women in the general population, the association between OPG levels and bone health is unclear, with conflicting reports on whether circulating OPG impacts upon bone mineral density (BMD) (Khosla et al., 2002; Piatek et al., 2013). In contrast, studies conducted among individuals with genetic disorders such as Paget's disease, which lead to OPG deficiency and severe osteopathy, support the notion that aberrant OPG levels may impair bone health (Whyte and Mumm, 2004). While understanding the role of the OPG/RANK pathway in the pathogenesis of *BRCA*-breast cancer is essential, whether its inherent dysregulation (and aberrantly lower OPG levels) also disrupts bone physiology in this population represents an additional concern.

Higher levels of osteopenia and osteoporosis has previously been reported in women with a *BRCA1* mutation compared to the general population (Garcia et al., 2015; Powell et al., 2018; Kotsopoulos et al., 2019); however, these studies included women with a personal cancer history and a prior risk-reducing bilateral salpingo-oophorectomy, which may independently contribute to bone loss (Kotsopoulos et al., 2019). It is conceivable that inherently lower OPG among women with a *BRCA1* or *BRCA2* mutation may also predispose them to diminished skeletal health. To our knowledge, there are no such studies that have been conducted in this population. Thus, the overall goal of the current study was to evaluate the association between serum OPG levels and two validated markers of bone health: 1) bone fracture risk score (FRAX) and 2) bone mineral density (BMD), among healthy women with a *BRCA1* mutation.

2. Materials and methods

2.1. Study population

Potentially eligible subjects included women with a pathogenic or likely pathogenic variant ('mutation' hereafter) in the *BRCA1* or *BRCA2* gene who were enrolled in an ongoing international, multi-center longitudinal study of high-risk women (previously described in detail) (https://www.sciencedirect.com/science/article/pii/S1470204506709834, n.d.). Briefly, participants complete a research questionnaire at the time of enrollment and every two years thereafter to collect detailed information on various exposures, general health, and medical history. The current study focused on women with a *BRCA1* mutation, who were at least 18 years of age, had a blood sample available for OPG quantification, completed at least one questionnaire, and had no personal history of cancer.

2.2. Data collection

The research questionnaires collected detailed information regarding medical history, medication use, screening, and preventive surgery as well as other lifestyle factors such as BMI, alcohol consumption, and smoking habits. For the current analysis, we used information collected from the questionnaire closest to the time of blood sample collection. All data was accessed on 29 September 2022 for research purposes. Authors did not have access to information that could identify individual participants.

2.3. Serum OPG quantification

Serum OPG was assessed using a commercial, enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol as previously described[4]. All serum samples were run in duplicate with a quality control sample on each plate. The average intra-assay coefficient of variation (CV) was calculated using the mean CV of duplicate samples within each plate. Samples with a CV >20 % were excluded. The concentration of OPG was subsequently calculated as the mean of the duplicate samples (each sample was adjusted for background signal and normalized to the blank wells) and then converted to the total OPG concentration upon comparison to the OPG standards provided by the manufacturer.

2.4. FRAX scores assessment

FRAX is a web-based algorithm that calculates an estimated country-specific 10-year risk of *hip* (FRAXhip) and *major* osteoporotic (FRAX-major) fractures, commonly referred to as FRAX scores (Kanis et al., 2017). The two FRAX scores were calculated for each participant using the University of Sheffield's online FRAX calculator (found at: https://www.sheffield.ac.uk/FRAX/tool.aspx). The FRAX tool incorporates information on various clinical risk factors such as age (years), sex (M/F), weight (kg), height (cm), history of fragility fracture (never/ever), current smoking (yes/no) and alcohol use (yes/no), glucocorticoid consumption (yes/no), history of rheumatoid arthritis (never/ever), and other conditions contributing to secondary osteoporosis (never/ever), including hyperthyroidism, diabetes type I, and premature menopause. Femoral neck BMD (which was not available for this population) is an *optional* variable, without which the algorithm can estimate FRAX scores. As outlined above, we used information from the questionnaire closest to the time of blood draw.

2.5. Bone mineral density assessment

There was a subset of women ($n = 50$) for whom lumbar spine BMD measurements were available which was previously quantified using dual x-ray absorptiometry (DXA) at the University Health Network, Women's College Hospital or referring centers in Ontario (Kotsopoulos et al., 2019). All BMD measurements were converted to Hologic equivalent values using standard reference formulas (Lu et al., 2001; Hui et al., 1997) and were reported in terms of g/cm^2 and by T-score at the lumbar spine.

2.6. Statistical analysis

The normality of FRAX and BMD were evaluated using the Shapiro-Wilks test. We initially categorized participants into high vs. low serum OPG using the median in the entire cohort; but also applied different cut-points including the 75th and 90th percentiles, as well as an OPG concentration of 119.2 pg/mL (SD = ± 89.4)which is the reported mean serum OPG in a healthy control population (Nava-Valdivia et al., 2021).

Given that FRAX scores were not distributed normally, the non-parametric Mann–Whitney *U* test was used to evaluate the association of serum OPG (pg/mL) with FRAXmajor and FRAXhip. We evaluated the association between serum OPG and FRAX in the entire cohort as well as stratified by menopausal status, given the established influence of age on both FRAX accuracy (Kanis et al., 2017) and circulating OPG levels (Liu et al., 2005).

In the supplemental analysis of serum OPG and lumbar spine BMD (g/cm^2), multivariate linear regression was used to evaluate the association of serum OPG (pg/mL) with lumbar spine BMD. The model was adjusted for age at blood draw (continuous), time between blood draw and DXA (years), BMI (continuous), personal history of breast cancer (yes/no) and selective estrogen receptor modulator (SERM) use (never/ever) (e.g., tamoxifen, raloxifene). Similarly, we evaluated the association in the entire cohort and further stratified by menopausal status. The adjusted least-squared means were back-transformed for ease of interpretation.

All analyses were conducted using SAS OnDemand for Academics. *P*-values were two-sided and considered statistically significant if $P \leq 0.05$.

3. Results

A total of 701 women with a *BRCA1* mutation were eligible for inclusion in the final analysis, which included 442 premenopausal and 259 postmenopausal women (Table 1). The average age at blood collection was 40.45 years (range 17–82) for the entire cohort, 33.23 years (range 17–57) for premenopausal and 52.78 years (range 36–82) for postmenopausal women. The mean serum OPG level across all women was 87.82 pg/mL (range 5.64–311.99) and varied significantly by menopausal status ($P < 0.0001$). Mean OPG was 82.15 pg/mL (range 9.11–311.99) for premenopausal women and 97.49 pg/mL (range 5.64–271.44) for postmenopausal women.

Fig. 1 summarizes the distribution of FRAX scores by serum OPG levels for all women combined and stratified by menopausal status.

Table 1
Baseline characteristics of *BRCA1* mutation carriers, all women combined and by menopausal status at blood draw.

Variable	All women ($n = 701$)	Premenopausal ($n = 442$)	Postmenopausal ($n = 259$)
Age at blood draw, mean (SD)	40.45 (12.23)	33.23 (7.01)	52.78 (8.94)
BMI (kg/m^2), mean (SD)	24.59 (4.83)	23.34 (4.38)	26.74 (4.82)
< 18.5 kg/m^2, n (%)	54 (7.70)	43 (9.73)	11 (4.24)
18.5–24.9 kg/m^2	375 (53.50)	285 (64.48)	90 (34.75)
25.0–29.9 kg/m^2	176 (25.11)	74 (16.74)	102 (39.38)
>30.0 kg/m^2	89 (12.70)	37 (8.37)	52 (20.08)
Bone disease[a], n (%)			
Never	667 (95.15)	436 (98.64)	231 (89.19)
Ever	34 (4.85)	6 (1.36)	28 (10.81)
Rheumatoid arthritis, n (%)			
Never	690 (98.43)	439 (99.32)	251 (96.91)
Ever	11 (1.57)	3 (0.68)	8 (3.09)
Current smoking, n (%)			
Never	565 (80.60)	349 (78.96)	216 (83.40)
Ever	136 (19.40)	93 (21.04)	43 (16.60)
Current alcohol consumption, n (%)			
Never	374 (53.35)	244 (55.20)	130 (50.19)
Ever	327 (46.65)	198 (44.80)	129 (49.81)
Oral contraceptive, n (%)			
Never	423 (60.34)	237 (53.62)	186 (71.82)
Ever	270 (38.52)	200 (45.25)	70 (27.03)
Postmenopausal HRT[b], n (%)			
Never	N/A	N/A	139 (53.67)
Ever			120 (46.33)
Menopause age, mean (SD)	N/A	N/A	45.59 (5.10)
Type of menopause, n (%)			
Surgical	N/A	N/A	154 (58.46)
Natural			102 (39.38)
Serum OPG (pg/mL), mean (SD)	87.82 (38.37)	82.15 (34.42)	97.49 (42.68)
FRAX 1: 10-year major osteoporotic fracture risk, mean (SD)	2.32 (1.20)	1.78 (0.39)	3.26 (1.50)
FRAX 2: 10-year hip fracture risk, mean (SD)	0.35 (0.56)	0.18 (0.12)	0.65 (0.84)

[a] Bone disease includes history of osteoporosis, osteoarthritis, and/or osteopenia reported closest to the date of blood draw.

[b] HRT use reflects ever use of hormone replacement therapy after surgical or natural menopause.

There was a significant positive correlation between FRAX and OPG among all women (FRAXmajor $\rho = 0.23$ $P < 0.001$, FRAXhip $\rho = 0.25$ $P < 0.001$) and by menopausal status (premenopausal: FRAXmajor $\rho = 0.14$ $P = 0.005$, FRAXhip $\rho = 0.15$ $P = 0.002$; postmenopausal: FRAXmajor $\rho = 0.17$ $P = 0.006$, FRAXhip $\rho = 0.2$- $P = 0.001$).

Table 2 summarizes the mean FRAX scores according to high vs. low serum OPG based on the median levels in the entire cohort and stratified by menopausal status. High OPG was associated with significantly higher FRAX scores in all women combined ($P < 0.0001$) and in the analysis stratified by menopausal status ($P < 0.05$). Findings were similar in the analysis using additional cut points to dichotomize the population into high vs. low OPG (Supplemental Table 1).

In our secondary analysis, we evaluated the association between circulation OPG and lumbar spine BMD in a subset of women with a *BRCA* mutation ($n = 50$). Table 3 summarizes the adjusted mean lumbar spine BMD in g/cm^2 (as well as the associated T-score) according to high vs. low levels of serum OPG among all women and stratified by menopausal status. Among all women, high OPG was associated with higher (although not statistically significant) lumbar spine BMD compared to those with low OPG (1.017 vs. 0.945; $P = 0.09$). Furthermore, lumbar spine BMD T-score was significantly higher in women with high vs. low OPG (−0.318 vs. -1.069; $P = 0.04$) (Table 3). The LS BMD and T-scores did not differ significantly by high vs. low OPG in the analyses stratified by menopausal status, although, this was based on small subgroups. Notably, although not significant, the same positive association was observed between both FRAX scores and serum OPG levels in this subgroup of women (FRAXmajor: 2.67 (low OPG) vs. 3.17 (high OPG); $P = 0.62$; FRAXhip: 0.20 (low OPG) vs. 0.25 (high OPG); $P = 0.51$) (Supplemental Table 2).

4. Discussion

Given the central role of the RANKL-OPG-signaling pathway in maintaining bone homeostasis (Simonet et al., 1997; Boyce and Xing, 2008; Boyce and Xing, 2007), we investigated whether there was an association between circulating OPG levels and bone health in women with a *BRCA1* mutation with inherently lower circulating OPG. Unexpectedly, increasing serum OPG was associated with a significant higher future risk of bone fracture (FRAX) in women with a germline *BRCA1* mutation. In contrast, lower serum OPG was associated with significantly lower BMD (the gold standard marker of bone disease (Williams and Sapra, 2023)) in a subset of women with bone density scan available. To our knowledge, this represents the first report of OPG levels and bone health in this specific population, and although inconclusive, our findings offer some preliminary insight into the mechanisms behind bone loss in women with a *BRCA1* mutation.

Recent data on aberrantly lower circulating levels of OPG (and higher RANKL) among women with an inherited *BRCA1* or *BRCA2* mutation suggest that dysregulation of this pathway may be implicated in breast cancer predisposition (Widschwendter et al., 2015). Widschwendter and colleagues were the first to report significantly lower mean circulating levels of OPG levels among 391 premenopausal *BRCA* mutation carriers compared to 782 non-carrier controls across the menstrual cycle (Widschwendter et al., 2015). Given this data, we posited that inherently lower OPG may also impact bone health in this population. To our surprise, the estimated 10-year risk of bone fracture was higher in women with higher serum OPG in the current study (FRAXmajor: 2.12 (low OPG) vs. 2.53 (high OPG); $P < 0.0001$; FRAXhip: 0.27 (low OPG) vs. 0.44 (high OPG); $P < 0.0001$).

Various factors may help explain this unexpected association. Studies have shown that femoral neck BMD (which was not available for this population) enhances FRAX predictability value (Kanis et al., 2009; Johansson et al., 2014). Thus, it may be that OPG levels are only relevant in predicting bone fracture risk when BMD measurements are available for inclusion in the calculation of the FRAX scores. Moreover, FRAX only calculates the risk of bone fracture for individuals over 40 (Siris et al.,

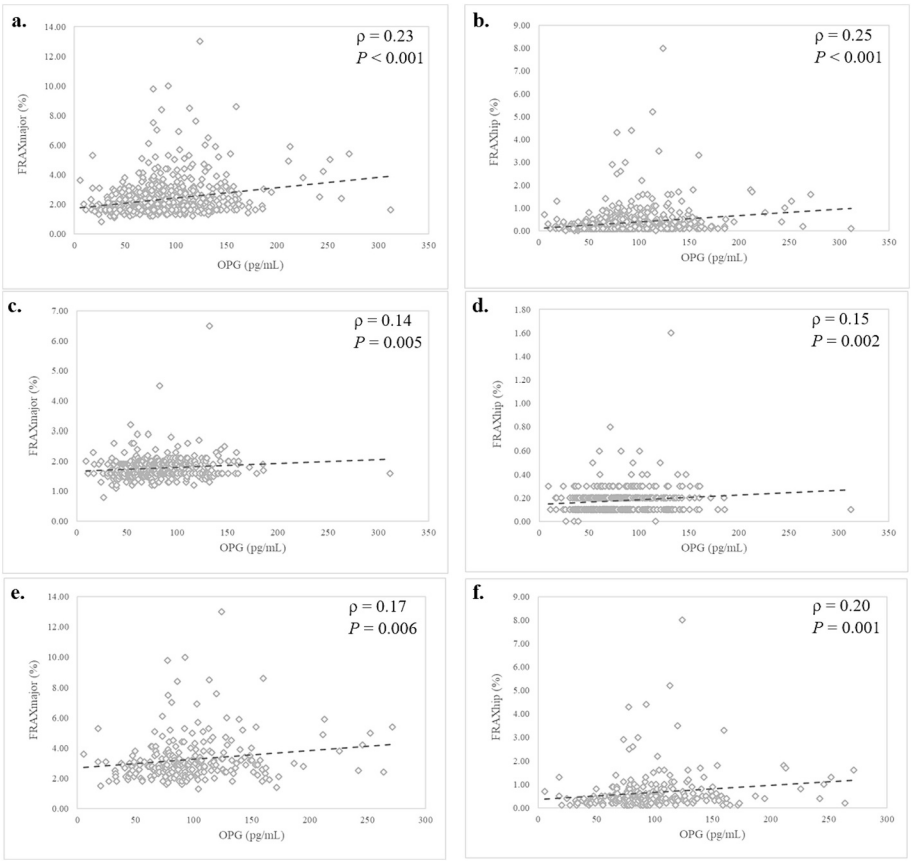

Fig. 1. Distribution of FRAX scores by serum OPG levels in *BRCA1* mutation carriers.
a. FRAXmajor[a] (%) and b. FRAXhip[b] (%) among all women; c. FRAXmajor[a] (%) and d. FRAXhip[b] (%) among premenopausal women; e. FRAXmajor[a] (%) and f. FRAXhip[b] (%) among postmenopausal women[c]
[a] *FRAXmajor = 10-year risk of developing major osteoporotic fracture (%)*
[b] *FRAXhip = 10-year risk of developing hip fracture (%)*
[c] *P-value and ρ-coefficient were calculated using Spearman's Rank Correlation.*

Table 2
Mean FRAX scores of *BRCA1* mutation carriers by high vs. low serum OPG levels, overall and by menopausal status.

OPG levels (pg/mL)[a]	n (%)	FRAXmajor[c] (%), mean (SD)	*P* value[b]	FRAXhip[d] (%), mean (SD)	*P* value[b]
All Women	701				
Low OPG	350 (50)	2.12 (0.94)		0.27 (0.38)	
High OPG	351 (50)	2.53 (1.38)	<0.0001	0.44 (0.70)	<0.0001
Premenopausal	442				
Low OPG	251 (57)	1.75 (0.34)		0.17 (0.09)	
High OPG	191 (43)	1.81 (0.44)	0.05	0.19 (0.14)	0.01
Postmenopausal	259				
Low OPG	99 (38)	3.07 (1.28)		0.54 (0.62)	
High OPG	160 (62)	3.38 (1.62)	0.10	0.72 (0.94)	0.03

[a] Serum OPG level was dichotomized at the median levels across the entire cohort (82.67 pg/mL).
[b] *P*-value was calculated using Mann–Whitney U test.
[c] FRAXmajor = 10-year risk of developing major osteoporotic fracture (%).
[d] FRAXhip = 10-year risk of developing hip fracture (%).

Table 3
Mean lumbar spine bone mineral density based on low vs. high serum OPG in a sub-population of women with a *BRCA1* or *BRCA2* mutation.

OPG (pg/mL)[a]	n (%)	LS BMD[b] (g/cm^2)[c], mean (SD)	*P* value[d]	LS BMD (T-score)[c], mean (SD)	*P* value[d]
All Women	50				
Low OPG	12 (24)	0.95 (0.16)		−1.07 (1.23)	
High OPG	38 (76)	1.017 (0.01)	0.09	−0.32 (1.19)	0.04
Premenopausal	32				
Low OPG	8 (25)	1.09 (0.15)		0.34 (1.23)	
High OPG	24 (75)	1.16 (0.09)	0.17	0.98 (1.42)	0.06
Postmenopausal	18				
Low OPG	5 (28)	0.91 (0.12)		−1.44 (0.76)	
High OPG	13 (72)	0.95 (0.11)	0.37	−0.93 (0.96)	0.08

[a] Serum OPG level was dichotomized at the median (98.20 pg/mL) across the entire cohort.
[b] LS BMD = lumbar spine bone mineral density.
[c] Adjusted for age at blood draw (continuous), time between blood draw and DXA (continuous), BMI (continuous), breast cancer history (yes/no), and ever use of SERMs tamoxifen or raloxifene (yes/no).
[d] *P*-value was calculated using multivariate linear regression.

2010). For those below 40, the default age input is 40, which may reduce the predictive power of this tool among young individuals. Lastly, declining bone health and/or the onset of bone disease are typically observed in postmenopausal women, increasing significantly in incidence with increasing age (Aspray and Hill, 2019). Therefore, given our relatively young study population (average age 40.5 years) with a smaller number of postmenopausal women (37 %), it is likely that most are not at risk of developing bone disease just yet, and the age-sensitivity of the FRAX algorithm has resulted in less accurate estimates for the younger majority. Future studies may be warranted to follow up on this cohort to investigate the association between OPG and FRAX once the women reach sufficient average age to be at a clinically relevant risk of bone fracture.

In contrast, we observed that lower OPG was significantly associated with lower lumbar spine BMD, the gold standard marker of bone disease (Williams and Sapra, 2023), in our supplementary analysis on a subpopulation. It is possible that inherently lower OPG levels leading to unabated RANK/RANKL signaling may contribute to accelerated bone loss in these women. Our findings have important implications for the management of bone health among *BRCA* mutation carriers especially given that this high-risk population is strongly advised to undergo risk-reducing bilateral salpingo-oophorectomy at a young age which puts them at risk for other health conditions including a decline in bone health (Garcia et al., 2015; Powell et al., 2018; Kotsopoulos et al., 2019). In a very recent report of women with a *BRCA1* or *BRCA2* mutation, we reported a significant post-oophorectomy BMD loss that was most apparent among women who were premenopausal at surgery (Kotsopoulos et al., 2019). HRT use mitigated some of the bone loss (Kotsopoulos et al., 2019). Thus, the management of cancer risk along with inherently lower OPG among *BRCA* mutation carriers may contribute to accelerated bone loss. Although HRT use did not impact FRAX scores among postmenopausal women in our study, women who underwent a bilateral salpingo-oophorectomy (and were more likely to use HRT) had lower FRAX scores compared to women who underwent natural menopause (and were less likely to use HRT) (data not shown).

The relationship between OPG and BMD has not been evaluated extensively and findings are inconsistent. In a large population-based study ($n = 2134$), Jorgensen et al., reported an inverse association between serum OPG levels and baseline BMD; however, a net increase in OPG levels after six years of follow-up was associated with a significant decline in BMD among postmenopausal women not using HRT only (Jørgensen et al., 2010). There was no association between a change in OPG and BMD in premenopausal women or postmenopausal women taking HRT (Jørgensen et al., 2010). In an analysis of 1379 postmenopausal women from the Framingham Offspring study, Samelson and colleagues reported a significant positive association between serum OPG and femoral BMD; however, they did not consider exogenous hormone use (Samelson et al., 2008). The latter findings are in line with our current report as well as other studies of postmenopausal women with (Stern et al., 2007) and without HRT use (Mezquita-Raya et al., 2005). The aforementioned studies were based on women that likely did not have a *BRCA* mutation, and thus, may not be directly applicable to our population of interest. Additional studies are warranted to confirm the association between OPG and BMD and may present an important avenue to maintain bone health with RANKL-inhibitors.

The receptor activator of nuclear factor κB (RANK), its cytokine ligand (RANKL), and the soluble receptor osteoprotegerin (OPG) form a pathway in the tumor necrosis factor (TNF) and TNF receptor superfamily (Rao et al., 2018; Kotsopoulos, 2018). These factors play a critical role in bone homeostasis, specifically in the regulation of bone resorption and remodeling (Simonet et al., 1997; Boyce and Xing, 2008; Boyce and Xing, 2007). OPG acts as a soluble decoy and, thus, antagonizes RANK/RANKL-mediated signaling (Nagy and Penninger, 2015; Kotsopoulos, 2018). Due to the pivotal role of this pathway in bone remodeling, its' dysregulation plays an important step in the development of osteoporosis as well as cancer-induced bone disease (Khosla et al., 2002; Piatek et al., 2013; Whyte and Mumm, 2004). As such, an anti-RANKL human monoclonal antibody (i.e., denosumab) is widely used to treat postmenopausal osteoporosis and prevent skeletal events in cancer patients undergoing treatment or those with bone metastases (Kotsopoulos, 2018; Lacey et al., 2012).

The role of estrogen deficiency, typically following menopause in women, on the development of osteoporosis has been well-established (Cheng et al., 2022). Although experimental data has shown that estrogen is associated with increased OPG expression, observational studies have consistently shown an increase in circulating OPG levels with both age and menopause (Khosla et al., 2002; Liu et al., 2005). The aforementioned is suggested to be a compensatory mechanism aimed at minimizing bone loss following menopause (Khosla et al., 2002; Liu et al., 2005). Although an established inhibitor of osteoclastogenesis, reports of circulating OPG levels and bone health among healthy women including BMD, bone turnover markers as well as subsequent risk of fracture have been conflicting and most have been conducted among postmenopausal women (Kearns et al., 2008). In the current study, most women were premenopausal (63 %) and average age was 40.5 years.

Our study had several limitations, notably the cross-sectional nature of this study, and thus, we cannot infer causality. OPG levels were only quantified once and, thus, may not be reflective of long-term OPG status. Nevertheless, Fortner et al., recently reported high reproducibility of OPG levels over time, including over one year ($r = 0.85$) and over 14 years ($r = 0.75$) (Fortner et al., 2017). Although we did not conduct a multivariate analysis to account for factors that may impact upon risk of bone fracture, the FRAX score integrates most important covariates, including age and BMI[16]. Strengths of our study included the use of blood samples and BMD assessments that were collected relatively close in time, the relatively low CVs demonstrating reliability of the OPG immunoassay, and the availability of detailed information from the medical history questionnaire allowing for the adjustment of potential covariates in our multivariate model of the BMD sub analysis.

In summary, this represents the first report of circulating OPG and markers of bone health specifically among women with a *BRCA1* mutation. Our observed and unexpected linear association between OPG and FRAX suggests that FRAX may not be a valuable marker of bone health in young women. We observed a linear relationship between increasing OPG and lumbar spine BMD, which suggests women with inherently low OPG may be at higher risk of developing bone disease. This warrants further evaluation in a larger number of participants. Additionally, further analyses may be conducted to evaluate whether the association between serum OPG and FRAX is modified by adding BMD measurements into fracture risk calculation. Importantly, the existence of an anti-RANKL inhibitor (i.e., denosumab) that may act to prevent bone loss, which has the potential to prevent *BRCA*-associated breast cancer, represents a very promising option for these high-risk women.

Ethics approval and consent to participate

The study was approved by the institutional ethics review boards of the host institutions and all study subjects provided written informed consent. The study was performed in accordance with the Declaration of Helsinki.

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CRediT authorship contribution statement

Aghaghia Mokhber: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elizabeth Hall:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Aleksandra Uzelac:** Investigation, Data curation. **Leonardo Salmena:** Resources, Data curation, Conceptualization. **Angela Cheung:** Resources, Data curation. **Jan Lubinski:** Resources, Data curation. **Steven A. Narod:** Writing – review & editing, Writing – original draft, Resources, Funding acquisition. **Joanne Kotsopoulos:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

All the authors named on this paper declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

Data availability

Data will be made available on request.

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